

(12)

EUROPEAN PATENT APPLICATION

(43) Date of publication: 07.05.2003 Bulletin 2003/19	(51) Int Cl.7: C12N 15/11, C12N 15/12, C12N 5/10, A01K 67/027, C07K 14/515, C12N 15/63, G01N 33/50, A61K 38/17, A61P 7/06, A61P 35/02
(21) Application number: 02021286.6	
(22) Date of filing: 19.09.2002	
(84) Designated Contracting States: AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LI LU MC NL PT SE SK TR Designated Extension States: AL LT LV MK RO SI	(72) Inventor: Esguerra, Camila V. 25337 Seeth-Ekholt (DE)
(30) Priority: 31.10.2001 US 335362 P	(74) Representative: Grund, Martin, Dr. et al Dr. Volker Vossius, Patentanwaltskanzlei, Geibelstrasse 6 81679 München (DE)
(71) Applicant: Mermaid Pharmaceuticals GmbH 20251 Hamburg (DE)	

(54) Use of angiopoietin-like 1 and angiopoietin-like 2 nucleic acids and proteins for the treatment of blood-related disorders and defects in vasculature.

(57) The present invention relates to methods of using ANGPTL1 and ANGPTL2 genes for altering clinical status. In particular, the present invention provides pharmaceutical compositions and antisense oligomers comprising polynucleotide sequences and amino acids sequences of the angiopoietin-like protein genes, ANGPTL1 and ANGPTL2. Furthermore, methods for screening for therapeutic agents, methods for determining whether a subject is at risk for a condition wherein

one or more cellular processes of erythroid growth, differentiation, maturation and survival is altered using polynucleotide sequences and amino acids sequences of the angiopoietin-like protein genes, ANGPTL1 and ANGPTL2. Moreover, the invention provides transgenic non-human animals lacking functional ANGPTL1 or ANGPTL2.

## Description

## ABSTRACT

**[0001]** The present invention relates to the use of nucleotide sequences of the ANGPTL1 and ANGPL2 (angiopoietin-like) genes, the amino acid sequences of the proteins encoded thereby, and derivatives (e.g., fragments) or analogs thereof for the treatment and diagnosis of blood-related disorders and defects in vasculature. In specific embodiments, the ANGPTL1 and ANGPL2 proteins are of human origin.

## BACKGROUND OF THE INVENTION

**[0002]** Receptor tyrosine kinases (RTKs) belong to the family of transmembrane receptors that, upon phosphorylation of specific tyrosine residues following ligand activation of the receptor, trigger activation of second messenger signaling systems to induce a biological response. A number of receptors that are found within this superfamily are those involved in normal vascular and hematopoietic development, such as flk1/KDR, flt1 flt4, TIE1 and TIE2 (Termann et al., 1992; Endo et al., 2001; Shibuya et al., 1990; de Vries et al., 1992; Millauer et al., 1993; Galland et al., 1993). More specifically, flk1 and flt1 belong to the VEGF receptor family of endothelium-specific receptor tyrosine kinases. In mice, flk1 and flt1 are expressed early in yolk sac mesoderm at the time of blood island formation, and in endothelial cells of later embryos and adults (Brier et al., 1995; Fong et al., 1995; Shalaby et al., 1995). Flt4, a receptor related to flt1, is also expressed in embryonic endothelial cells and later becomes restricted to lymphatic vessels (Kaipainen et al., 1995).

**[0003]** TIE1 and TIE2 belong to the family of receptor tyrosine kinases with immunoglobulins and epidermal growth factor homology domains, whose homologues have been cloned in other species: murine TEK and zebrafish Z tie-1 and -2 (Davis et al., 1996; Maisonpierre et al., 1997; Neufeld et al., 1999; Valenzuela et al., 1999; Lyons et al., 1998). Expression of these receptors is restricted to differentiated vascular endothelial cells, as well as a subset of hematopoietic stem cells (Mustonen and Alitalo, 1995; Lyons et al., 1998; Claesson-Welsh, 1999; Hamaguchi et al., 1999).

**[0004]** Several members of the angiopoietin (ANGPTs or ANGs) family of proteins have been shown to interact with TIE2 receptor, either as agonists or antagonists (Davis et al., 1996; Maisonpierre et al., 1997; Valenzuela et al., 1999; Pham et al., 2001), to mediate remodeling of primary vascular networks into large blood vessels, a process termed angiogenesis (Risau, 1997; Liang et al., 2001). Not surprisingly, ANGs and TIE2 receptor have also been implicated in playing a critical role in promoting proliferation of hematopoietic progenitor cells (Takakura et al., 1998; Huang et al., 1999; Hattori et al., 2001).

**[0005]** Recently, several angiopoietin-related or -like proteins have been identified based on their structural homology to the angiopoietins family of proteins (Conklin et al., 1999; Kim et al., 1999a; Kim et al., 1999b); angiopoietin-like 1 (ANGPTL1), angiopoietin-like or -related 2 (ANGPTL2 or ARP2), angiopoietin-like or -related 3 (ANGPTL3 or ARP3), angiopoietin-like 4 (ANGPTL4) and angiopoietin-like 5 (ANGPTL5).

**[0006]** Both ANGPTL1, formally angiopoietin-3 (Kim et al., 1999a), and ANGPTL2 (Kim et al., 1999b) molecules include the characteristic coiled-coil ( $\alpha$ -helical rich) segment, which helps to multimerize the fibrinogen-like domain; both domains are conserved in the angiopoietin family of proteins. The fibrinogen-like domain is a highly conserved receptor-binding domain, which determines whether an angiopoietin molecule will behave as an agonist or antagonist (Xu et al., 1996; Procopio et al., 1999). The gene expression patterns for ANGPTL1 and ANGPTL2 differ in human adult tissue: ANGPTL1 is ubiquitously expressed (Kim et al., 1999a) whereas ANGPTL2 is expressed specifically in the spleen, stomach, heart and small intestine. Furthermore, ANGPTL1 is highly expressed in vascularized human adult tissues (Kim et al., 1999a).

**[0007]** Mitogenic assays for ANGPTL1 in human umbilical vein endothelial cells (HUVECs) show that ANGPTL1 protein is secreted by endothelial cells, however, it cannot stimulate endothelial cell proliferation (Kim et al., 1999a), suggesting that this mitogen is only required for the maintenance of differentiated stage of endothelial cells in most human adult tissue. Furthermore, recombinant ANGPTL2 has been shown to induce sprouting of cultured human umbilical vein endothelial cells (HUVECs) (Kim et al. 1999b), but it also does not stimulate endothelial cell proliferation. This effect has also been observed for angiopoietin-1 (ANG1), an agonist of the TIE2 receptor (Davis et al., 1996). Since ANGPTL1 and ANGPTL2 hold a significant degree of amino acid sequence homology to ANG1, it is possible that both mitogenic factors induce sprouting of endothelial cells by activating the phosphatidylinositol 3'-kinase/Akt signal transduction pathway via the TIE receptors (Kontos et al., 1998; Kim et al., 2000). Furthermore, ANG1 has recently been shown to be an apoptosis survival factor for endothelial cells (Kwak et al., 1999), suggesting that ANGPTL1, and possibly ANGPTL2 may also be an apoptosis survival factor for endothelial cells during vascular remodeling, similar to that of ANG1. However, neither of these molecules (ANGPTL1 and ANGPTL2) has been shown to bind TIE1 or TIE2 (Kim et al., 1999b), suggesting that a similar receptor/signal transduction pathway is being utilized to mediate vascular remodeling.

**[0008]** Furthermore, since TIE/TEK receptors are expressed in hemangioblasts (Hamaguchi et al., 1999), and both

ANGs and TIE2 receptor have been implicated in playing a critical role in promoting proliferation of hematopoietic progenitor cells (Takakura et al., 1998; Huang et al., 1999; Hattori et al., 2001), angiopoietin-like proteins, via a similar receptor, may play a similar role in stimulating these multipotent cells to differentiate into hematopoietic stem cells during development.

## SUMMARY OF THE INVENTION

**[0009]** The present invention provides novel evidence for the role of the ANGPTL1 and ANGPTL2 genes in the cellular processes of erythroid growth, differentiation, maturation, and survival.

**[0010]** Generally, the present invention relates to the use of nucleotide sequences of the ANGPTL1 and ANGPTL2 (angiopoietin-like or related) genes, the amino acid sequences of their encoded proteins, and derivatives (e.g., fragments) or analogs thereof. In specific embodiments, the ANGPTL1 and ANGPTL2 proteins are human proteins.

**[0011]** In certain preferred embodiments, said nucleotide sequences used according to the present invention are operably linked to an expression control sequence. The invention also provides host cells, including bacterial, yeast, insect and mammalian cells, transformed with such nucleotide sequences.

**[0012]** The present invention provides a pharmaceutical composition comprising a polynucleotide sequence selected from the group consisting of:

- a. the polynucleotide sequence of SEQ ID NO:2;
- b. fragments and derivatives of the polynucleotide sequence of SEQ ID NO:2;
- c. the polynucleotide sequence of SEQ ID NO:3;
- d. fragments and derivatives of the polynucleotide sequence of SEQ ID NO:3.

**[0013]** The composition may further comprise a pharmaceutically acceptable carrier.

**[0014]** Compositions comprising a protein biological activity are also provided. In preferred embodiments the composition comprises an amino acid sequence selected from group consisting of:

- (a) the amino acid sequence of SEQ ID NO: 7;
- (b) fragments and derivatives of the amino acid sequence of SEQ ID NO: 7;
- (c) the amino acid sequence of SEQ ID NO: 8;
- (d) fragments and derivatives of the amino acid sequence of SEQ ID NO: 8; and the protein being substantially free from other mammalian proteins. Such compositions may further comprise a pharmaceutically acceptable carrier.

**[0015]** Methods are also provided for treating of blood-related disorders and defects in vasculature, which comprises administering to a mammalian subject a therapeutically effective amount of a composition comprising:

(a) a polypeptide selected from the group consisting of:

- a. the amino acid sequence of SEQ ID NO: 7;
- b. fragments or derivatives of the amino acid sequence of SEQ ID NO: 7;
- c. the amino acid sequence of SEQ ID NO: 8;
- d. fragments or derivatives of the amino acid sequence of SEQ ID NO: 8; and/or

(b) a polynucleotide sequence selected from the group consisting of:

- a. the polynucleotide sequence of SEQ ID NO: 2;
- b. fragments or derivatives of the polynucleotide sequence of SEQ ID NO: 2;
- c. the polynucleotide sequence of SEQ ID NO: 3;
- d. fragments or derivatives of the polynucleotide sequence of SEQ ID NO: 3.

**[0016]** The composition may further contain a pharmaceutically acceptable carrier.

**[0017]** Furthermore, the present invention relates to a method for screening a library of test molecules or compounds to identify at least one molecule or compound which specifically binds and/or interacts with polypeptides selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO: 7;
- (b) fragments and derivatives of the amino acid sequence of SEQ ID NO: 7;

(c) the amino acid sequence of SEQ ID NO: 8;

(d) fragments and derivatives of the amino acid sequence of SEQ ID NO: 8; and the library of molecules or compounds is selected from the group consisting of DNA molecules, peptides, agonists, antagonists, antibodies, immunoglobulins and pharmaceutical agents.

5

**[0018]** In one aspect, the invention provides a pharmaceutical composition comprising angiopoietin-like 1 (ANGPTL1) or angiopoietin-like 2 (ANGPTL2) protein, a fragment or a derivative thereof.

**[0019]** Preferably, this composition comprises a polypeptide, which is at least 55% identical with SEQ ID NO: 7, or SEQ ID NO: 8, a fragment or derivative thereof.

10

**[0020]** Furthermore, the invention is directed to methods for treating a blood-related disorder or a defect in vasculature, comprising administering to a patient a therapeutically effective amount of polypeptide comprising ANGPTL1 or ANGPTL2, or a fragment or a derivative thereof. Preferably, the polypeptide is at least 55% identical with SEQ ID NO: 7 or SEQ ID NO: 8, fragment or derivative thereof.

15

**[0021]** A further aspect of the invention encompasses a pharmaceutical composition comprising a polynucleotide sequence encoding ANGPTL1 or ANGPTL2, a fragment, or a derivative thereof. Preferably, the polynucleotide sequence is at least 70% identical with a polynucleotide sequence which encodes SEQ ID NO: 7, or SEQ ID NO: 8, a fragment or a derivative thereof or the polynucleotide sequence is at least 70% identical with a polynucleotide sequence selected from the group comprising of SEQ ID NO: 2, or SEQ ID NO: 3. In a preferred embodiment the composition further comprises a suitable carrier. Preferably, the polynucleotide sequence is operably linked to an expression control sequence. In one embodiment of the invention the polynucleotide sequence, which is operably linked to an expression control sequence is in a host cell. This host cell is preferably a yeast, insect, teleost, amphibian or mammalian cell.

20

**[0022]** Furthermore, the invention is directed to methods for treating a blood-related disorder or a defect in vasculature, comprising administering to a patient a therapeutically effective amount of polynucleotide sequence encoding ANGPTL1 or ANGPTL2, or a fragment or a derivative thereof. Preferably, the polynucleotide sequence is at least 70% identical with a polynucleotide sequence which encodes SEQ ID NO:7 or SEQ ID NO:8, or a fragment or a derivative thereof or is at least 70% identical with a polynucleotide sequence selected from the group consisting of SEQ ID NO: 2 or SEQ ID NO:3.

25

**[0023]** Preferably the polynucleotide sequence is in a delivery complex and the delivery complex can be a sterol, lipid or virus.

30

**[0024]** In a further aspect, the invention provides methods of screening for a therapeutic agent for the treatment of blood-related disorders or a defect in vasculature comprising: contacting ANGPTL1 polypeptide, ANGPTL2 polypeptide, fragment or derivative thereof with a test compound or molecule or a library of test molecules or compounds, under conditions to allow specific binding and/or interaction; and detecting the level of specific binding and/or interaction, wherein an alteration in the level of interaction relative to a control indicates a potential therapeutic activity.

35

**[0025]** Furthermore, the invention is directed to methods of screening for a therapeutic agent for the treatment of blood-related disorders or a defect in vasculature comprising: contacting a reporter construct under the control of ANGPTL1 or ANGPTL2 promoter with a test molecule or compound or a library of test molecules or compounds, under conditions to allow specific binding and/or interaction; and detecting the level of expression of the reporter construct, wherein an alteration in the level of expression to a control indicates a potential therapeutic activity.

40

**[0026]** Preferably, the test molecule or test compound or library of test molecules or compounds is selected from the group consisting of DNA molecules, peptides, agonists, antagonists, monoclonal antibodies, immunoglobulins and pharmaceutical agents.

45

**[0027]** In a further embodiment the invention relates to methods for the treatment of blood-related disorders or defects in vasculature in a mammalian, preferably human patient comprising: providing a composition that comprises test molecules or compounds of therapeutic agents identified to bind or interact with angiopoietin-like polypeptides or modulate the level of angiopoietin-like expression; AND administering a therapeutically effective amount of said composition to the patient. Preferably, the composition comprising the test molecules or compounds also comprises a suitable carrier.

50

**[0028]** In a preferred embodiment, the blood-related disorder treated according to the methods of the present invention is leukemia or anemia.

**[0029]** A further aspect of the invention is a method of modulating proliferation and/or differentiation or cell death of target cells comprising administering a biologically active amount of ANGPTL1 or ANGPTL2 polypeptide or polynucleotide to said target cells. Preferably, said target cells are hematopoietic stem cells, erythroid or endothelial cells.

55

**[0030]** One embodiment of invention provides a method for determining whether a subject is at risk of developing or has a blood related disease or a defect in vasculature caused by ANGPTL1 or ANGPTL2 comprising determining the angiopoietin-like allele status of a subject; an altered angiopoietin-like allele status compared to a control indicating that said subject is at risk of developing or has a blood-related disease or a defect in vasculature caused by angiopoietin-like proteins.



**[0031]** Furthermore, the invention relates to a kit for identifying a subject at risk of developing a blood-related disease or a defect in vasculature or has a blood-related disease or a defect in vasculature caused by angiopoietin-like comprising means for measuring the level of an angiopoietin-like encoding polynucleotide sequence in a sample of cells of the subject and instructions to use the kit.

**[0032]** In another embodiment of the invention provides a transgenic non-human animal, which does not produce functional ANGPTL1 and/or ANGPTL2 protein or which produces sub-optimal levels of ANGPTL1 and/or ANGPTL2 protein or which overproduces the ANGPTL1 or ANGPTL2 protein. Preferably, the animal comprises a mutation of ANGPTL1 or ANGPTL2 genes. More preferably said mutation is a homozygous disruption. This mutation can be generated by insertion, deletion, or a substitution mutation. In a preferred embodiment said mutation is generated by gene targeting, gene trap integration, or mutagenesis. Preferably, the mutation has occurred in an exon, intron, regulatory region or splicing site of the ANGPTL1 and/or ANGPTL2 genes. In a further embodiment said animal exhibits a blood-related disease or a defect in vasculature and preferably said animal is a mammal, amphibian, teleost or insect.

**[0033]** The invention further provides antisense oligomers, wherein said antisense oligomers are complementary to mRNA encoding ANGPTL1 or ANGPTL2, and are from 6 to 50 nucleotides in length. Preferably, the antisense oligomers are complementary to the translation initiation site of ANGPTL1 or ANGPTL2. More preferably said antisense oligomers are complementary to the polynucleotide sequence of SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4, or the antisense oligomer comprises the nucleotide sequence SEQ ID NO: 5 or SEQ ID NO: 6. In a preferred embodiment the binding of said oligomer to said mRNA is effective in altering transcription or translation of said mRNA in a host cell expressing said mRNA. Preferably, the host cell is a bacterial, yeast, insect, or vertebrate cell. Furthermore, the antisense oligomer is preferably in a delivery complex and said delivery complex can be a sterol, lipid, or virus.

**[0034]** In one preferred embodiment the oligomers comprise: a) at least one modified base moiety which is selected from the group of 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), -5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine, AND/OR at least one modified sugar moiety selected from the group including but not limited to arabinose, 2-fluoroarabinose, xylulose, and hexose, AND/OR at least one modified phosphate backbone selected from the group consisting of a phosphorothioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, a formacetal or analog thereof, as well as  $\alpha$ -DNA, 2'-O-methyl RNA, and morpholine backbone.

## DESCRIPTION OF FIGURES

### **[0035]**

Figure 1. Nucleotide sequence of zfEST encoding a partial polypeptide belonging to the angiopoietin Y1 of proteins.

Figure 2. Results of BLASTP search using longest ORF of zfEST (AW174181)

Figure 3. Multiple sequence alignment of vertebrate angiopoietin-like family proteins.

Figure 4. cDNA sequence of zebrafish cDNA clone similar to angiopoietin Y1.

Figure 5. cDNA sequence of human angiopoietin-like 1 precursor.

Figure 6. cDNA sequence of human angiopoietin-like 2 precursor.

Figure 7. cDNA sequence of mouse angiopoietin-related 2 precursor.

Figure 8. antisense oligonucleotide against zebrafish angiopoietin-like

- a) antisense oligonucleotide sequences against zebrafish angiopoietin-like 1 precursor
- b) antisense oligonucleotide sequences against zebrafish angiopoietin Y1

Figure 9. amino acid sequence for:

- a) human angiopoietin-like 1 precursor.
- b) human angiopoietin-like 2 precursor.

Figure 10. amino acid sequences of motifs found in angiopoietin-like molecules:

- a) The amino acid sequence of the calcium-binding motif,
- b) The amino acid sequence of the fibrinogen beta and gamma chains C-terminal domain,
- c) The amino acid sequence of the secretory signal sequence.

Figure 11. Analysis of hemoglobin (o-dianisidine) stained zebrafish embryos after morpholino (MO) knockdown.

Figure 12. Whole mount in situ hybridization analysis

- a) GATA-1 marker expression
- b) Flt-4 marker expression
- c) Flk-1 marker expression
- d) Fli-1 marker expression
- e) Imo-2 marker expression

## DETAILED DESCRIPTION

**[0036]** It is to be understood that the present invention is not limited to the particular materials and methods described or equipment, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention, which will be limited only by the appended claims.

**[0037]** It should be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a host cell" includes a plethora of such host cells, and a reference to "an antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

**[0038]** Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art. Although any materials and methods, or equipment similar or equivalent to those specifically described herein can be used to practice or test the present invention, the preferred equipment, materials and methods are described below. All publications mentioned herein are cited for the purpose of describing and disclosing the cell lines, protocols, reagents and vectors which are reported in the publications and which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

## DEFINITIONS

**[0039]** The term "derivative" refers to the chemical modification of a polypeptide sequence, a polynucleotide sequence, or antisense oligonucleotide sequence. Chemical modifications of a polynucleotide sequence include, but are not limited to, for example, the replacement of hydrogen by an alkyl, acyl, or amino group.

**[0040]** The term "biologically active," is used interchangeably with the terms "biological activity", "bioactivity" or "activity" and for the purposes herein means an effector or antigenic function that is directly or indirectly performed by an angiopoietin-like polypeptide (whether in its native or denatured conformation), or by any subsequence thereof. Effector functions include phosphorylation (kinase activity) or activation of other molecules, induction of differentiation, mitogenic or growth promoting activity, induction of apoptosis, signal transduction, immune modulation, DNA regulatory functions and the like, whether presently known or inherent. Antigenic functions include possession of an epitope or antigenic site that is capable of cross-reacting with antibodies raised against a naturally occurring or denatured angiopoietin-like polypeptide or fragment thereof. Accordingly, a biological activity of an angiopoietin-like protein can be phosphorylation (kinase activity) of other molecules. A biological activity of an angiopoietin-like protein can also be modulation of cell proliferation and/differentiation. A target cell can be, e.g., hematopoietic stem cell, erythroid progenitor cell, an endothelial cell, or a cancer cell.

**[0041]** A "composition comprising a given nucleotide sequence" or a "composition comprising a given amino acid sequence" refer broadly to any composition containing the given polynucleotide or amino acid sequence (polypeptide). The composition may comprise a dry formulation or an aqueous solution. Compositions comprising polynucleotide

sequences encoding ANGPTL or fragments of ANGPTL may be employed as hybridization probes. The probes may be stored in freeze-dried form and may be associated with a stabilizing agent such as a carbohydrate. In hybridizations, the probe may be deployed in an aqueous solution containing salts (e.g., NaCl), detergents (e.g., sodium dodecyl sulfate; SDS), and other components (e.g., Denhardt's solution, dry milk, salmon sperm DNA, etc.).

**[0042]** A coding sequence is "operably linked to" another coding sequence when RNA polymerase will transcribe the two coding sequences into a single mRNA which is then translated into a single polypeptide having amino acids derived from both coding sequences. The coding sequences need not be contiguous to one another so long as the expressed sequence is ultimately processed to produce the desired protein. The term "reporter construct" encompasses a target gene linked in frame to another sequence to provide a coding unit whose product is easily assayed. Examples of reporter genes include neo, geo, hygro and puro.

**[0043]** The phrases "nucleotide sequence", "oligonucleotide sequence" or "polynucleotide sequence" refer to a nucleotide, oligonucleotide, polynucleotide, or any fragment thereof. These phrases also refer to DNA or RNA of genomic or synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide polynucleotide sequence (PNA), or to any DNA-like or RNA-like material. In this context, "fragments" refers to those polynucleotide sequence sequences which, when translated, would produce polypeptides retaining some functional characteristic, e.g., antigenicity, or structural domain characteristic, e.g., ATP-binding site, of the full-length polypeptide.

**[0044]** As is well known, genes for a particular polypeptide may exist in single or multiple copies within the genome of an individual. Such duplicate genes may be identical or may have certain modifications, including nucleotide substitution, additions or deletions, all of which still code for polypeptides having substantially the same activity. The term "polynucleotide sequence encoding an angiopoietin-like polypeptide" may thus refer to one or more genes within a particular individual. Moreover, certain differences in nucleotide sequences may exist between individual organisms, which are called alleles. Such allelic differences may or may not result in differences in the amino acid sequence of the encoded polypeptide yet still encode a protein with the same biological activity.

**[0045]** The term "oligomer/s" is used herein to mean oligonucleotides and related structural compounds.

**[0046]** The term "transcriptional regulatory sequence" or "regulatory region" or "expression control sequence" includes promoters, enhancers and other expression control elements.

**[0047]** "Cells", "host cells", "target cells" or "recombinant host cells" are terms used interchangeably herein and refer to a cell which has been transformed or transfected, or is capable of transformation or transfection by an exogenous polynucleotide sequence. It is understood that such terms refer not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progenys may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

**[0048]** As used herein, a "target molecule" is a molecule with which said polypeptide binds or interacts with, for example, a molecule associated with the internal surface of a cell membrane or a cytoplasmic molecule.

**[0049]** A "delivery complex" shall mean a targeting means (e.g., a molecule that results in higher affinity binding of an antisense oligomer, protein, polypeptide, or peptide to a target cell surface and /or increased cellular uptake by a target cell). Examples of targeting means include: sterols (e.g. cholesterol), lipids (e.g. a cationic lipid, virosome or liposome), viruses (e.g. adenovirus, adeno-associated virus, or retrovirus). Preferred complexes are sufficiently stable *in vivo* to prevent significant uncoupling prior to internalization by the target cell. However, the complex is cleavable under appropriate conditions within the cell so that the polynucleotide, protein, polypeptide, or peptide is released in a functional form.

**[0050]** "A blood-related disorder" encompasses any disease, ailment, or malady that is the result of defects during hematopoiesis, erythropoiesis, hemoglobin synthesis and/or heme production. These include, but are not limited to, a failure of hematopoietic stem cells, erythroid-myeloid progenitor cells or lymphoid progenitor cells to differentiate; abnormal differentiation of hematopoietic stem cells, erythroid-myeloid progenitor cells or lymphoid progenitor cells; absence or decreased production of hemoglobin; or inhibited heme synthesis.

**[0051]** "A defect in vasculature" relates to an alteration in normal blood vessel formation within any given animal. In the context of the invention, this includes, but is not limited to, a lack or significant decrease of blood vessel formation, an overabundance of blood vessels, invasive blood vessel formation (amount of vessels formed is normal, however formation follows no defined pattern), abnormally slow or rapid (e.g. hemangionomas) vasculogenesis and/or angiogenesis, arrested vasculogenesis and/ or angiogenesis, or abnormal blood vessel wall formation (e.g. structural defects).

**[0052]** "Antisense oligomer" refers to an antisense molecule or anti-gene agent that comprises an oligomer of at least about 5 nucleotides, (or any derivatives thereof) in length. In embodiments an antisense oligomer comprises at least 6, 7, 8, 9, 10, 12, 15, 18 or 20 nucleotides.

**[0053]** A "transgenic animal" refers to any animal, preferably a non-human mammal, bird, fish or an amphibian, in which one or more of the cells of the animal contain heterologous polynucleotide sequence introduced by way of human

intervention, such as by transgenic techniques well known in the art. The polynucleotide sequence is introduced into the cell, directly or indirectly by introduction into a precursor of the cell, by way of deliberate genetic manipulation, such as by microinjection or by infection with a recombinant virus. This polynucleotide sequence may be integrated within a chromosome (stable transgenic), or it may be extra-chromosomally replicated (transient transgenic). In the typical transgenic animals described herein, the transgene causes cells to express a recombinant form of one of the angiopoietin-like proteins, e.g. either agonistic or antagonistic forms. Moreover, "transgenic animal" also includes those recombinant animals in which gene disruption of one or more angiopoietin-like genes is caused by human intervention, including both recombination and antisense techniques.

**[0054]** The term "does not produce functional ANGPTL1 and/or ANGPTL2 protein" denotes the lack of translation of an effective ANGPTL1 and/or ANGPTL2 protein.

**[0055]** The term "produces sub-optimal levels of ANGPTL1 and/or ANGPTL2" encompasses the translation level of ANGPTL1 and/or ANGPTL2 protein, which is insufficient to produce an effective concentration of ANGPTL1 and/or ANGPTL2. Preferably, the level is reduced at least by 50%, more preferably by 70% and most preferably by 95 %.

**[0056]** The term "homozygous disruption of the ANGPTL1 and/or ANGPTL2 genes" relates to an identical mutation in both ANGPTL1 and/or ANGPTL2 alleles.

**[0057]** The term "mutation" refers to a change of one or more nucleotide pairs of a DNA molecule.

**[0058]** The term "insertion" is directed to a mutation identified by the presence of one or more additional base pairs in the DNA.

**[0059]** The term "deletion" relates to a mutation generated by removal of a sequence of DNA (one or more base pairs), the regions on either side being joined together.

**[0060]** The term "substitution mutation" is directed to a nucleotide change. The substitution mutation can result in an amino acid change or can introduce a premature translation stop codon. Furthermore, a substitution mutation can affect splicing or expression of the gene when occurring in the sites necessary for splicing or regulatory regions of the gene.

**[0061]** The term "gene targeting" relates to a type of homologous recombination that occurs when a fragment of genomic DNA is introduced into a cell and that fragment locates and recombines with homologous sequences.

**[0062]** The term "gene trap integration" is directed to insertion of a vector, which comprises a reporter gene and which is activated upon its insertion within an active transcription unit, into a chromosomal site.

**[0063]** The term "mutagenesis" denotes a chemical or physical treatment that changes nucleotides in the genome of an organism. An example of a chemical mutagenesis is N-ethyl-N-nitrosurea (ENU) mutagenesis.

**[0064]** The term "exon" encompasses a segment of a gene which is decoded to give a mRNA product or a mature RNA product. Individual exons may contain protein coding DNA and/or non-coding DNA.

**[0065]** The term "intron" denotes non-coding DNA, which separates neighboring exons in a gene. During gene expression introns, like exons are transcribed into RNA but the transcribed intron sequences are subsequently removed by RNA splicing and are not present in mRNA.

**[0066]** The term "regulatory region" relates to the nucleotide sequence, which comprises regions, which are necessary for the regulation of the transcription of the gene. These regions comprise e.g. promoters and enhancers and they can be located in the 5' region of the exons or in introns.

**[0067]** The term "splicing site" encompasses the nucleotides at a) the end of an exon and the beginning of the intron and at b) the end of the intron and beginning of the exon that are required for the joining of two exons and removal of an intron during the processing of a primary transcript to a functional mRNA.

**[0068]** The term "angiopoietin-like gene allele status" denotes the state of the DNA sequence in both alleles of an angiopoietin-like gene, i.e. whether the angiopoietin-like gene contains a mutation and whether this mutation is heterozygous or homozygous.

**[0069]** The terms "specific binding" or "specifically binding" refer to the interaction between two proteins, a protein and a peptide, two peptides, a protein or peptide and an agonist, an antibody, or an antagonist. The interaction is dependent upon the presence of a particular structure of the protein, e.g., docking site of specific kinases, phosphorylated amino acids, the antigenic determinant or epitope, recognized by the binding molecule. For example, if the amino acid sequence of protein A docking site is recognized by the protein docking site of protein B; in a reaction containing protein A, labeled protein B and unlabeled protein B, the unlabeled protein B will reduce the amount of labeled protein B being able to bind protein A. The term "interaction" relates to direct or indirect binding or alteration of expression at the polynucleotide or polypeptide level.

**[0070]** The term "agonist" refers, in one embodiment, to a molecule which, when bound to ANGPTL, increases or prolongs the duration of the effect of angiopoietin-like proteins. Agonists may include proteins, polynucleotide sequences, carbohydrates, or any other molecules which bind to and modulate the effect of angiopoietin-like proteins.

**[0071]** The term "antagonist" refers, in one embodiment, to a molecule or compound which, when bound to ANGPTL1 and/or ANGPTL2 decreases receptor-binding activity of an angiopoietin-like protein. A preferred angiopoietin-like antagonist inhibits the interaction between an angiopoietin-like protein and another molecule, e.g. (tyrosine kinase re-



ceptors). Alternatively, an angiopoietin-like antagonist can be a compound which modulates the expression or activity of a protein which is located downstream of angiopoietin-like or which interacts with an angiopoietin-like protein. An angiopoietin-like antagonist can also be a molecule or compound which decreases the amount or the duration of the effect of the biological or immunological activity of angiopoietin-like proteins. A preferred angiopoietin-like antagonist inhibits the interaction between an angiopoietin-like protein and another molecule, e.g. tyrosine kinase receptors. Antagonists may include proteins, polynucleotide sequences, carbohydrates, antibodies, or any other molecule that exerts these effects.

**[0072]** The term "stringent conditions" refers to conditions which permit hybridization between polynucleotides and the claimed polynucleotides. Stringent conditions can be defined by salt concentration, the concentration of organic solvent, e.g., formamide, temperature, and other conditions well known in the art. In particular, stringency can be increased by reducing the concentration of salt, increasing the concentration of formamide, or raising the hybridization temperature.

**[0073]** A "chimeric protein" or "fusion protein" is a fusion of a first amino acid sequence encoding an angiopoietin-like polypeptide with a second amino acid sequence defining a domain (e.g. polypeptide portion) foreign to and not substantially homologous with any domain of the angiopoietin-like protein.

**[0074]** A chimeric protein may present a foreign domain which is found (albeit in a different protein) in an organism which also expresses the first protein, or it may be an "interspecies", "intergenic", e.g. fusion of protein structures expressed by different kinds of organisms. In general, a fusion protein can be represented by the general formula X-ANGPTL-Y, wherein ANGPTL represents a portion of the protein which is derived from the angiopoietin-like protein, and X and Y are independently absent or represent amino acid sequences which are not related to one of the angiopoietin-like sequences in an organism, including naturally occurring mutants.

**[0075]** The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject.

## DETAILED DESCRIPTION OF THE INVENTION

### Angiopoietin-like protein therapeutics

**[0076]** The first aspect of the present invention provides pharmaceutical compositions comprising polypeptides selected from the group consisting of SEQ ID Nos. 7 or 8, fragments and derivatives thereof in combination with a pharmaceutically suitable carrier. In a preferred embodiment, therapeutically effective amounts of said polypeptides, fragments and derivatives will be used in the treatment of blood disorders and defects in vasculature. In an even more preferred embodiment, the blood-related disorders are leukemia and anemia.

**[0077]** In another embodiment of the invention, pharmaceutical compositions are provided comprising polynucleotides selected from the group consisting of SEQ ID Nos. 2 or 3, fragments and derivatives thereof, in combination with a pharmaceutically acceptable carrier. In one aspect, the complement of said polynucleotides may be used in situations in which it is desirable to block transcription of mRNA. In particular, cells may be transformed with sequences complementary to polynucleotides selected from SEQ ID Nos. 2 or 3, which encode angiopoietin-like proteins. Thus, complementary molecules or fragments may be used to modulate angiopoietin-like activity, or to achieve regulation of gene function. Such technology is well known to those skilled in the art. In a preferred embodiment, therapeutically effective amounts of said polynucleotides, fragments and derivatives will be used in the treatment of blood disorders and defects in vasculature. In an even more preferred embodiment, the blood-related disorders are leukemia and anemia.

**[0078]** Expression vectors derived from retroviruses, adenoviruses, or herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of polynucleotide sequences to the targeted organ, tissue (e.g. bone marrow), or cell population (hematopoietic stem cells, erythroid cells or endothelial cells). Methods which are well known to those skilled in the art, can be used to construct vectors to express nucleic acid sequences complementary to the polynucleotides encoding angiopoietin-like proteins (see Current Protocols In Molecular Biology, John Wiley & Sons, N.Y. 3.16.1-3.16.11).

**[0079]** An additional embodiment of the invention relates to the administration of a pharmaceutical composition, in conjunction with a pharmaceutically acceptable carrier, for any of the therapeutic effects discussed above. Such pharmaceutical compositions may comprise one or more agents selected from the group consisting of angiopoietin-like polypeptides, angiopoietin-like encoding polynucleotides, agonist and antagonists of angiopoietin-like proteins, inhibitors of angiopoietin-like proteins, and antibodies to angiopoietinlike proteins. The compositions may be administered alone or in combination with at least one other agent, such as a stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical carrier including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs, or hormones.

**[0080]** Pharmaceutical compositions used according to the present invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual or rectal.

**[0081]** Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

**[0082]** For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

**[0083]** In a preferred embodiment, pharmaceutical compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of a therapeutically effective dose is well within the capability of those skilled in the art. For any pharmaceutical composition, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., leukemia cell lines, or in animal models such as zebrafish, mice, rats, or rabbits. An animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

**[0084]** A therapeutically effective dose refers to that amount of active ingredient, for example angiopoietin-like polypeptides, angiopoietin-like encoding polynucleotides, agonist and antagonists of angiopoietin-like proteins, inhibitors of angiopoietin-like proteins, or antibodies to angiopoietin-like proteins, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals, known to those skilled in the art. Pharmaceutical compositions which exhibit large therapeutic indices are preferred.

**[0085]** The pharmaceutical composition of the present invention may be manufactured in a manner that is known to those skilled in the art, e.g. by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

**[0086]** The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, and succinic acid. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms.

#### Screening for therapeutics

**[0087]** The invention provides a method, also referred to herein as a "screening assay", for identifying candidates, test compounds, test molecules, or agents (e.g. peptides, peptidomimetics, small molecules or other drugs) which bind to a polypeptide selected from the group consisting of SEQ ID Nos. 7 or 8, fragment or derivative thereof, or have a stimulatory or inhibitory effect on, for example, expression or activity of said polypeptides.

**[0088]** In one embodiment, the above-mentioned polypeptide has at least one bioactivity of an angiopoietin-like polypeptide, such as the ability to activate tyrosine kinase receptors. Proteins or peptides capable of interacting directly or indirectly with the angiopoietin-like proteins, fragments or derivatives thereof, can be identified by various methods, e.g., methods based on binding assays (see references on: yeast-2-hybrid Bemis et al. (1995) *Methods Cell Biol.* 46: 139-51, Fields et al. (1994) *Trends Genet.* 10:286-92, Topcu and Borden (2000) *Pharm. Res.* 17:1049-1055; yeast 3 hybrid: Zhang et al. (1999) *Methods Enzymol.* 306:93-113; GST-pull-downs as in Palmer et al. (1998) *EMBO J.* 17: 5037-5047; and phage display as in Scott and Smith (1990) *Science.* 249:386-390).

**[0089]** In other preferred embodiments, the bioactivity of an angiopoietin-like polypeptide is to modulate proliferation and/or differentiation or cell death of specific target cells, e.g., hematopoietic stem cells, erythroid cells or endothelial cells. Assays for determining that an angiopoietin-like polypeptide has at least one bioactivity of an angiopoietin-like protein are described infra.

**[0090]** In one embodiment, the invention provides assays for screening test compounds or molecules which bind to or modulate the activity of the biologically active form of a polypeptide selected from the group consisting of SEQ ID Nos. 7 and 8, fragments and derivatives thereof. The test compounds according to the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries, aptially addressable parallel solid phase or solution phase libraries, synthetic library methods requiring deconvolution, the "one-bead-one-compound" library method, and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Bindseil et al. (2001) *Drug Discov. Today* 6:840-847; Grabley et al. (2000) *Ernst Schering Res. Found. Workshop.* pp. 217-252; Houghten et al. (2000) *Drug Discov. Today* 5:276-285; and Rader (2001) *Drug Discov. Today* 6:36-43).

**[0091]** Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:6909-13; Erb et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:11422-25; Gallop

et al. (1994) J. Med. Chem. 37:1233-1251; and Gordon et al. (1994) J. Med. Chem. 37:1385-401.

**[0092]** Libraries of compounds may be presented in solution (e.g., Houghten (1992) Biotechniques. 13:412-421), or on beads (Lam (1991) Nature. 354:82-84), chips (Fodor (1993) Nature. 364:555-556), bacteria (U. S. Patent No. 5,223,409), spores (Patent NOS. 5,571,698; 5,403,484; and 5,223,409), plasmids (Cull et al. (1992) Proc. Natl. Acad. Sci. USA. 89:1865-1869) or phage (Scott and Smith (1990) Science. 249:386-390; Devlin (1990) Science. 249:404-406; Cwirla et al. (1990) Proc. Natl. Acad. Sci. USA. 87:6378-6382; and Felici (1991) J. Mol. Biol. 222:301-310).

**[0093]** In one embodiment, the assay is a cell-based assay in which a cell expresses a biologically active polypeptide selected from the group consisting of SEQ ID Nos. 7 or 8, a fragment or derivative thereof. The expressed polypeptide is contacted with a test compound and the ability of the test compound to bind to the polypeptide is determined. The cell can, for example, be a yeast cell, insect cell, teleost cell, an amphibian cell, or a cell of mammalian origin. Determining the ability of the test compound or molecule to bind to the polypeptide can be accomplished, for example, by coupling the test compound or test molecule with a radioisotope (e.g.  $^{125}\text{I}$ ,  $^{35}\text{S}$ ,  $^{14}\text{C}$ , or  $^3\text{H}$ ) or enzymatic (e.g. horse-radish peroxidase, alkaline phosphatase, or luciferase) label such that binding of the test compound or molecule to the biologically active polypeptide, fragment, or derivative thereof can be determined by detecting the labelled compound in the complex. Methods of labelling and detecting interactions of test compounds with cytosolic proteins are known to those skilled in the art. In a preferred embodiment, the assay comprises contacting a cell, which expresses a biologically active polypeptide selected from the group consisting of SEQ ID Nos. 7 or 8, fragment, or derivative thereof, with a known compound which binds the polypeptide to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with said polypeptide, wherein determining the ability of the test compound to interact with said polypeptide is compared to a control.

**[0094]** In another embodiment, the assay is a cell-based assay comprising contacting a cell expressing a biologically active polypeptide selected from the group consisting of SEQ ID Nos. 7 or 8, fragment, or derivative thereof, with a test compound or test molecule and determining the ability of the test compound or molecule to modulate (e.g., stimulate or inhibit) the activity of said polypeptide. Determining the ability of the test compound or molecule to modulate the activity of said polypeptide can be accomplished, for example, by determining the ability of said polypeptide to bind to or interact with a target molecule.

**[0095]** Determining the ability of a biologically active polypeptide selected from the group consisting of SEQ ID Nos. 7 or 8, fragment or derivative thereof, to bind to or interact with a target molecule can be accomplished by one of the methods described above for determining direct binding. A target molecule can be a biologically active polypeptide selected from SEQ ID. Nos. 7 or 8, fragment, or derivative thereof, or another polypeptide or protein. For example, a target molecule can be a component of a signal transduction pathway which facilitates transduction of an extracellular signal, a second intercellular protein which has catalytic activity, a protein which regulates transcription of specific genes, or a protein that initiates protein translation. Determining the ability of a biologically active polypeptide selected from the group consisting of SEQ ID Nos. 7 or 8, fragment or derivative thereof, to bind to or interact with a target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (e.g., intracellular  $\text{Ca}^{++}$ , diacylglycerol, IP3, etc.), detecting catalytic/enzymatic activity of the target on an appropriate substrate, detecting the induction (via a regulatory element that may be responsive to said polypeptide) of a reporter gene operably linked to a nucleic acid encoding a detectable marker, e.g., luciferase, or detecting a cellular response, for example, cellular differentiation, or cell proliferation.

**[0096]** In yet another embodiment, the assay of the present invention is a cell-free assay comprising contacting a biologically active polypeptide selected from the group consisting of SEQ ID Nos. 7 or 8, a fragment or derivative thereof with a test compound or molecule, and determining the ability of the test compound or molecule to bind to said polypeptide. Binding of the test compound or molecule to said polypeptide can be determined either directly or indirectly as described above. In a preferred embodiment, the assay includes contacting said polypeptide with a known compound, which binds the polypeptide to form an assay mixture. The assay mixture is contacted with a test compound or molecule, and the determination of the ability of the test compound or molecule to interact with the polypeptide is based on competitive binding/inhibition kinetics of the test compound or molecule and known compounds for said polypeptide. Methods of detecting of competitive binding of two molecules for the same target, wherein the target is a polypeptide selected from the group consisting of SEQ ID. Nos. 7 or 8, fragments or derivatives thereof are known to those skilled in the art.

**[0097]** In another embodiment, the assay is a cell-free assay comprising contacting a biologically active polypeptide selected from the group consisting of SEQ ID Nos. 7 or 8, fragment, or derivative thereof with a test compound or molecule, and determining the ability of the test compound or molecule to modulate (e.g., stimulate or inhibit) the activity of said polypeptide. Determining the ability of the test compound or molecule to modulate the activity of said polypeptide can be accomplished, for example, by determining the ability of said polypeptide to bind to a target molecule by one of the methods described above for determining direct binding. In an alternative embodiment, determining the ability of the test compound or molecule to modulate the activity of said polypeptide can be accomplished by determining the



ability of said polypeptide to further modulate a target molecule.

**[0098]** In embodiments of the above assay methods of the present invention, it may be desirable to immobilize either the polypeptide selected from the group consisting of SEQ ID Nos. 7 or 8, fragment, or derivative thereof, or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound or molecule to said polypeptide, or interaction of said polypeptide with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase fusion proteins or glutathione-S-transferase fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical; St. Louis, MO) or glutathione derivatized microtitre plates, which are then combined with the test compound or the test molecule and either the non-adsorbed target protein or a biologically active polypeptide selected from the group consisting of SEQ ID Nos. 7 or 8, fragment or derivative thereof. The mixture is then incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or microtitre plate wells are washed to remove any unbound components and complex formation is measured either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of binding or activity of the polypeptide can be determined using standard techniques.

**[0099]** Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either the biologically active polypeptide selected from the group consisting of SEQ ID Nos. 7 or 8, fragment or derivative thereof, or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin.

**[0100]** In another embodiment, modulators of expression of an ANGPTL polypeptide are identified in a method in which a cell is contacted with a candidate compound and the expression of the selected mRNA or protein (i. e., the mRNA or protein corresponding to a biologically active polypeptide (SEQ ID Nos. 7 or 8; SEQ ID Nos. 2 or 3) in the cell is determined. In a preferred embodiment, the cell is an animal cell. Even more preferred, the cell can be derived from insects, fish, amphibians, mice, rats, or humans. The level of expression of the selected mRNA or protein in the presence of the candidate compound is compared to the level of expression of the selected mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of expression of the ANGPTL polypeptide based on this comparison. For example, when expression of the selected mRNA or protein is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of the selected mRNA or protein expression. Alternatively, when expression of the selected mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of the selected mRNA or protein expression. The level of the selected mRNA or protein expression in the cells can be determined by methods described herein.

**[0101]** In another embodiment, an angiopoietin-like therapeutic can also be identified by using a reporter assay in which the level of expression of a reporter construct, under the control of an angiopoietin-like protein promoter, is measured in the presence or absence of a test compound. An angiopoietin-like promoter can be isolated by screening a genomic library with an angiopoietin-like cDNA which preferably contains the 5' end of the cDNA. A portion of the angiopoietin-like promoter, typically from about 50 to about 500 base pairs long is then cloned upstream of a reporter gene, e.g., a luciferase gene, in a plasmid. This reporter construct is then transfected into cells, e.g., erythroid cells or endothelial cells. Transfected cells are then distributed into wells of a multi-well plate and various concentrations of test compounds are added to the wells. After several hours of incubation, the level of expression of the reporter construct is determined according to methods known in the art. A difference in the level of expression of the reporter construct in transfected cells incubated with the test compound relative to transfected cells incubated without the test compound will indicate that the test compound is capable of modulating the expression of the angiopoietin-like protein gene and is thus an angiopoietin-like therapeutic.

**[0102]** In one embodiment of the invention, angiopoietin-like therapeutics can be used for treating various diseases, including blood diseases, diseases or conditions associated with defects in vasculature, specifically leukemia and anemia. In addition, based at least in part on the similarity of the amino acid sequence and structure between the various angiopoietin-like proteins, angiopoietin-like therapeutics can be used to treat diseases or conditions associated with an aberrant angiopoietin-like activity or an aberrant angiopoietin-like activity other than angiopoietin-like activity.

**[0103]** This invention further pertains to novel agents identified by the above-described screening assays and uses thereof for treatments as described herein.

**55**     Diagnostics

**[0104]** Another aspect of the present invention relates to diagnostic assays for determining the expression of an angiopoietin-like polypeptide or polynucleotide encoding an angiopoietin-like protein, and/or the activity of said polypep-



5 tide, in the context of a biological sample (e.g., blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant expression or activity of an angiopoietin-like polypeptide, e.g. a blood-related disorder or a defect in vasculature. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with aberrant expression or activity of an angiopoietin-like polypeptide. For example, mutations in a gene encoding an angiopoietin-like protein can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with aberrant expression or activity of an angiopoietin-like polypeptide.

10 **[0105]** A method for detecting the presence or absence of an angiopoietin-like polypeptide or polynucleotide encoding an angiopoietin-like polypeptide, e.g., the amino acid sequences selected from the group consisting of SEQ ID Nos. 7 and 8, in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting said polypeptide or polynucleotide (e.g., mRNA, genomic DNA) such that the presence of said polypeptide or polynucleotide is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. That is, the detection method of the invention can be used to detect mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of an angiopoietin-like polypeptide include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. *In vitro* techniques for detection of genomic DNA include Southern hybridizations. Furthermore, *in vivo* techniques for detection of an angiopoietin-like polypeptide include introducing into a subject a labeled antibody directed against the polypeptide.

20 **[0106]** In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample contains mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a peripheral blood sample isolated by conventional means from a subject.

25 **[0107]** In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting an angiopoietin-like polypeptide or mRNA or genomic DNA encoding an angiopoietin-like polypeptide, such that the presence of the polypeptide or mRNA or genomic DNA encoding the angiopoietin-like polypeptide is detected in the biological sample, and comparing the presence of said polypeptide or mRNA or genomic DNA encoding an angiopoietin-like polypeptide in the control sample with the presence of the polypeptide or mRNA or genomic DNA encoding the polypeptide in the test sample.

30 **[0108]** In yet another embodiment, the polynucleotide sequences selected from the group consisting of SEQ ID Nos. 2 and 3, are used as templates for the generation of probes and primers designed for use in identifying and/or cloning angiopoietin-like homologs in other cell types, e.g. from other tissues, as well as angiopoietin-like homologs from other mammalian organisms. Probes and primers of the invention can also be used to determine the identity of an angiopoietin-like allele and/or the presence or absence of one or more mutations in an angiopoietin-like gene of a subject. In a preferred embodiment, a probe or primer of the invention can be used to determine whether a subject has or is at risk of developing a disease or condition associated with a specific angiopoietin-like allele, such as an allele carrying a mutation.

35 **[0109]** In a preferred embodiment, in the present invention a probe/primer comprising a substantially purified oligonucleotide is used, which oligonucleotide comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, preferably about 25, more preferably about 40, 50 or 75 consecutive nucleotides of sense or antisense sequence selected from the group consisting of SEQ ID Nos. 2 and 3, or naturally occurring mutants thereof. For instance, primers based on the polynucleotide sequence represented in SEQ ID Nos. 2 and 3 can be used in PCR reactions to clone angiopoietin-like homologs, e.g. specific angiopoietin-like alleles. Such primers are preferably selected in a region that does not share significant homology to other genes, e.g., other angiopoietin genes. Likewise, probes based on angiopoietin-like sequences can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In preferred embodiments, the probe further comprises a label group attached thereto and able to be detected, e.g. the label group is selected from amongst radioisotopes, fluorescent compounds, enzymes, and enzyme co-factors.

40 **[0110]** Such probes can also be used as a part of a diagnostic test kit for identifying cells or tissue which mis-express an angiopoietin-like protein, such as by measuring the level of an angiopoietin-like encoding polynucleotide sequence in a sample of cells from a patient; e.g. detecting angiopoietin-like mRNA levels or determining whether a genomic angiopoietin-like gene has been mutated or deleted. Briefly, nucleotide probes can be generated from an angiopoietin-like gene that facilitate histological screening of intact tissue and tissue samples for the presence (or absence) of angiopoietin-like protein encoding transcripts. Similar to the diagnostic uses of anti-angiopoietin-like antibodies, the use of probes directed to angiopoietin-like messages, or to genomic angiopoietin-like sequences, can be used for both predictive and therapeutic evaluation of allelic mutations which might be manifested in, for example, blood disorders (e.g. anemia) (e.g. unwanted cell growth) or abnormal differentiation of tissue. Used in conjunction with immunoassays

as described herein, the oligonucleotide probes can help facilitate the determination of the molecular basis for a developmental disorder which may involve some abnormality associated with expression (or lack thereof) of an angiopoietin-like protein. For instance, variation in polypeptide synthesis can be differentiated from a mutation in a coding sequence. Also within the scope of the invention are kits for determining whether a subject is at risk of developing a disease or condition caused by or contributed by an aberrant angiopoietin-like bioactivity and/or which is associated with one or more specific angiopoietin-like alleles. In a preferred embodiment, the kit can be used for determining whether a subject is at risk of developing a blood disorder, e.g. anemia or leukemia.

#### Model systems

**[0111]** Cell- and animal-based systems may be used in a variety of applications, for example, to further characterize angiopoietin-like genes and proteins. In addition, such assays may be utilized as part of screening strategies designed to identify compounds that are capable of ameliorating disease symptoms. Thus, the animal- and cell-based models may be used to identify drugs, pharmaceuticals, therapies and interventions that may be effective in treating disease.

#### Animal-based systems

**[0112]** One aspect of the present invention concerns transgenic animals comprised of cells (of that animal) which contain a transgene and which preferably (though optionally) express an exogenous angiopoietin-like protein in one or more cells in the animal. Preferably said transgenic animal is a mammal, amphibian, teleost or insect. More preferably said transgenic animal is a mouse, rat or a zebrafish. An angiopoietin-like transgene can encode the wild-type form of the protein, or can encode homologs thereof, including both alleles of angiopoietin-like genes, agonists and antagonists, as well as antisense constructs. In preferred embodiments, the expression of the transgene is restricted to specific subsets of cells, tissues or developmental stages utilizing, for example, cis-acting sequences that control expression in the desired pattern. In the present invention, such mosaic expression of a angiopoietin-like protein can be essential for many forms of lineage analysis and can additionally provide a means to assess the effects of, for example, lack of angiopoietin-like expression which might grossly alter development in small patches of tissue within an otherwise normal embryo. In a preferred embodiment, the invention provides transgenic zebrafish having an allele of the angiopoietin-like gene and the zebrafish can be used, for example, to determine the effect of this specific angiopoietin-like allele on differentiation and/development of specific tissues and cell types (e.g. hematopoietic stem cells, erythroid progenitor cells, vasculature). Tissue-specific regulatory sequences and conditional regulatory sequences can be used to control expression of the transgene in certain spatial patterns. Moreover, temporal patterns of expression can be provided by, for example, conditional recombination systems or prokaryotic transcriptional regulatory sequences. Genetic techniques that allow for the expression of transgenes via site-specific genetic manipulation *in vivo* are known to those skilled in the art. The transgenic animals of the present invention all include within their cells a transgene, which alters the phenotype of the "host cell" with respect to regulation of cell growth, death and/or differentiation. It is noted that those skilled in the art are able to incorporate specific transgene sequences into organisms utilizing any of the methods and materials selected from the group of the cre/loxP recombinase system of bacteriophage P1 (Lakso et al. (1992) PNAS. 89:6232-6236; Orban et al. (1992) PNAS. 89:6861-6865) or the FLP recombinase system of *S. cerevisiae* (O'Gorman et al. (1991) Science. 251:1351-1355; PCT publication WO 92/15694). Similar conditional transgenes can be provided using prokaryotic promoter sequences that require prokaryotic proteins to be simultaneously expressed in order to facilitate expression of an angiopoietin-like transgene. Exemplary promoters and the corresponding trans-activating prokaryotic proteins are given in U.S. Pat. No. 4,833,080. Furthermore, the transgenic animals of the present invention preferably exhibit alteration of one or more cellular processes of erythroid growth, differentiation, maturation and survival (e.g. conditions resembling anemia or leukemia), alteration in blood vessel development and formation, establishment of stable blood vessel architecture.

**[0113]** Moreover, expression of the conditional transgenes can be induced by gene therapy-like methods wherein a gene encoding the trans-activating protein, e.g. a recombinase or a prokaryotic protein, is delivered to the tissue and caused to be expressed, such as in a cell-type specific manner. By this method, an angiopoietin-like transgene could remain silent into adulthood until "turned on" by the introduction of the trans-activator.

**[0114]** In an exemplary embodiment, the "transgenic non-human animals" of the invention are produced by introducing transgenes into the germ-line of a non-human animal. Embryonal target cells at various developmental stages can be used to introduce transgenes. Different methods are used depending on the stage of development of the embryonal target cell. The specific line(s) of any animal used to practice this invention are selected for general good health, good embryo yields, good pronuclear visibility in the embryo, and good reproductive fitness. In addition, the haplotype is a significant factor. For example, when transgenic zebrafish are to be produced, strains such as Tübingen, AB, TL, or Florida wildtype are often used. The line(s) used to practice this invention may themselves be transgenics, and/or may be knockouts (i.e., obtained from animals which have one or more genes partially or completely sup-

pressed).

**[0115]** In one embodiment, the transgene construct is introduced into a single-cell stage zebrafish embryo. The use of zygotes as a target for gene transfer has a major advantage in that in most cases the injected DNA will be incorporated into the host gene before the first cleavage (Brinster et al. (1985) Proc. Natl. Acad. Sci. USA 82:4438-4442). As a consequence, all cells of the transgenic animal will carry the incorporated transgene. This will in general also be reflected in the efficient transmission of the transgene to offspring of the founder since 50% of the germ cells will harbor the transgene.

**[0116]** Introduction of the transgene nucleotide sequence into the zebrafish embryo may be accomplished by any means known in the art, but preferably by microinjection (Meng et al. (1999) Methods Cell. Biol. 60:133-148; Westerfield (2000) The Zebrafish Book). Following introduction of the transgene nucleotide sequence into the embryo, the embryo may be incubated for varying periods of time. Incubation to maturity is within the scope of this invention.

**[0117]** In addition to similar biological considerations, physical ones also govern the amount (e.g., volume) of exogenous genetic material that can be added to the zygote or to the genetic material. The volume of exogenous genetic material inserted will not exceed about 10 nanoliters. The physical effects of addition must not be so great as to physically destroy the viability of the zygote. The biological limit of the number and variety of DNA sequences will vary depending upon the particular zygote and functions of the exogenous genetic material and will be readily apparent to one skilled in the art, because the genetic material, including the exogenous genetic material, of the resulting zygote must be biologically capable of initiating and maintaining the differentiation and development of the zygote into a functional organism.

**[0118]** The number of copies of the transgene constructs, which are introduced into the zygote, is dependent upon the total amount of exogenous genetic material added and will be the amount which enables the genetic transformation to occur. Theoretically only one copy is required, however, generally, numerous copies are utilized, for example, 1,000-20,000 copies of the transgene construct, in order to insure that one copy is functional. As regards the present invention, there will often be an advantage to having more than one functioning copy of each of the inserted exogenous DNA sequences to enhance the phenotypic expression of the exogenous DNA sequences.

**[0119]** Any technique which allows for the addition of the exogenous genetic material into nucleic genetic material can be utilized so long as it is not destructive to the cell, nuclear membrane or other existing cellular or genetic structures. The exogenous genetic material is preferentially inserted into the nucleic genetic material by microinjection. Microinjection of cells and cellular structures is known and is used in the art.

**[0120]** Alternative or additional methods for evaluating the presence of the transgene include, without limitation, suitable biochemical assays such as enzyme and/or immunological assays, histological stains for particular marker or enzyme activities, flow cytometric analysis, and the like. Analysis of the blood may also be useful to detect the presence of the transgene product in the blood, as well as to evaluate the effect of the transgene on the levels of various types of blood cells and other blood constituents.

**[0121]** Progeny of the transgenic animals may be obtained by mating the transgenic animal with a suitable partner, or by in vitro fertilization of eggs and/or sperm obtained from the transgenic animal. Where mating with a partner is to be performed, the partner may or may not be transgenic and/or a knockout; where it is transgenic, it may contain the same or a different transgene, or both. Alternatively, the partner may be a parental line. The progeny may be evaluated for the presence of the transgene using methods described above, or other appropriate methods.

**[0122]** Retroviral infection can also be used to introduce a transgene into a non-human animal. The developing non-human embryo can be cultured *in vitro* to the blastocyst stage. During this time, the blastomeres can be targets for retroviral infection (Jaenich (1976) PNAS. 73:1260-1264). Alternatively, infection can be performed at a later stage. Virus or virus-producing cells can be injected into the blastocoele (Jahner et al. (1982) Nature. 298:623-628). The viral vector system used to introduce the transgene is typically a replication-defective retrovirus carrying the transgene (Jahner et al. (1985) PNAS. 82:6927-6931; Van der Putten et al. (1985) PNAS. 82:6148-6152). Following the methods and material described previously, those skilled in the art will be able to retrovirally introduce nucleic acids into a non-human embryo to generate transgenic non-human animal.

**[0123]** A third type of target cell for transgene introduction is the embryonal stem cell (ES). ES cells are obtained from pre-implantation embryos cultured in vitro and fused with embryos (Evans et al. (1981) Nature. 292:154-156; Bradley et al. (1984) Nature. 309:255-258; Gossler et al. (1986) Proc.Natl. Acad. Sci. USA 83: 9065-9069; and Robertson et al. (1986) Nature. 322:445-448). Transgenes can be efficiently introduced into the ES cells by DNA transfection or by retrovirus-mediated transduction. For review see Jaenisch (1988) Science. 240:1468-1474.

**[0124]** Gene targeting in embryonic stem cells is in fact a scheme contemplated by the present invention as a means for disrupting an angiopoietin-like gene function through the use of a targeting transgene construct designed to undergo homologous recombination with one or more angiopoietin-like genomic sequences. The targeting construct can be arranged so that, upon recombination with an element of an angiopoietin-like gene, a positive selection marker is inserted into (or replaces) coding sequences of the targeted angiopoietin-like gene. The inserted sequence functionally disrupts the angiopoietin-like gene, while also providing a positive selection trait. Generally, the embryonic stem cells



(ES cells) used to produce the knockout animals will be of the same species as the knockout animal to be generated. Thus for example, mouse embryonic stem cells will usually be used for generation of a knockout mouse. Embryonic stem cells are generated and maintained using methods well known to the skilled artisan such as those described by Doetschman et al. ((1985) J. Embryol. Exp. Morphol. 87:27-45). The cells are cultured and prepared for knockout construct insertion using methods well known to the skilled artisan. Insertion of the knockout construct into the ES cells can be accomplished using a variety of methods well known in the art including for example, electroporation, microinjection, and calcium phosphate treatment. A preferred method of insertion is microinjection. For insertion, the knockout construct is added to the ES cells under appropriate conditions for the insertion method chosen, as is known to the skilled artisan. Where more than one construct is to be introduced into the ES cell, each knockout construct can be introduced simultaneously or one at a time.

**[0125]** Screening of the above-described cells can be accomplished using a variety of methods such as antibiotic resistance, Southern blot analysis of ES cell genomic DNA, PCR or enzymatic analysis (for example using specific substrate). One skilled in the art will be familiar with other useful markers and the means for detecting their presence in a given cell. All such markers are contemplated as being included within the scope of the teaching of this invention.

**[0126]** After suitable ES cells containing the knockout construct in the proper location have been identified, the cells can be inserted into an embryo. Insertion may be accomplished in a variety of ways known to the skilled artisan, however a preferred method is by microinjection. For microinjection, about 10-30 cells are collected into a micropipette and injected into embryos that are at the proper stage of development to permit integration of the foreign ES cell containing the knockout construct into the developing embryo. For instance, as the appended Examples describe, the transformed ES cells can be microinjected into blastocytes.

**[0127]** Other means of identifying and characterizing the knockout offspring are available. For example, Northern blots can be used to probe the mRNA for the presence or absence of transcripts encoding either the gene knocked out, the marker gene, or both. In addition, Western blots can be used to assess the level of expression of the angiopoietin-like gene knocked out in various tissues of the offspring by probing the Western blot with an antibody against the particular angiopoietin-like protein, or an antibody against the marker gene product, where this gene is expressed. Finally, *in situ* analysis (such as fixing the cells and labeling with antibody) and/or FACS (fluorescence activated cell sorting) analysis of various cells from the offspring can be conducted using suitable antibodies to look for the presence or absence of the knockout construct gene product.

**[0128]** Yet other methods of making knockout or disruption transgenic animals are also generally known. (See, e.g. Koster et al. (2001) Dev. Biol. 233:329-346; Huang et al. (2001) Mol. Cell. Endocrinol. 177:117-124; Long et al. (1997) Development 124:4105-4111; Motoike et al. (2000) Genesis 28:75-81.

**[0129]** Recombinase dependent knockouts can also be generated, e.g. by homologous recombination to insert target sequences, such that tissue specific and/or temporal control of inactivation of an ANGPTL-gene can be controlled by recombinase sequences.

**[0130]** Animals containing more than one knockout construct and/or more than one transgene expression construct are prepared in any of several ways. The preferred manner of preparation is to generate a series of mammals, each containing one of the desired transgenic phenotypes. Such animals are bred together through a series of crosses, backcrosses and selections, to ultimately generate a single animal containing all desired knockout constructs and/or expression constructs, where the animal is otherwise congenic (genetically identical) to the wild type except for the presence of the knockout construct(s) and/or transgene(s).

**[0131]** The transgenic animals produced in accordance with the present invention will include exogenous genetic material. As set out above, the exogenous genetic material will, in certain embodiments, be a DNA sequence that results in the production of an angiopoietin-like protein (either agonistic or antagonistic), an antisense transcript, or an angiopoietin-like mutant. Further, in such embodiments the sequence will be attached to a transcriptional control element, e.g., a promoter, which preferably allows the expression of the transgene product in a specific type of cell.

#### Antisense oligomers

**[0132]** A further aspect of the invention relates to "antisense" therapy. As used herein, "antisense" therapy refers to the administration or *in situ* generation of oligomer molecules or their derivatives which specifically hybridize (e.g. bind) under cellular conditions, with the cellular mRNA and/or genomic DNA encoding one or more angiopoietin-like proteins so as to inhibit expression of that protein(s), e.g. by inhibiting transcription and/or translation. The binding may be by classical base pair complementarity, or, for example, in the case of binding to DNA duplexes, through specific interactions in the major groove of the double helix. In general, "antisense" therapy refers to the range of techniques generally employed in the art, and includes any therapy that relies on specific binding of oligomers to target polynucleotide sequences.

**[0133]** An antisense construct of the present invention can be delivered, for example, as an expression plasmid which, when transcribed in the cell, produces RNA which is complementary to at least a unique portion of the cellular



mRNA which encodes an angiopoietin-like protein. Alternatively, the antisense construct is an oligomer probe that is generated *ex vivo* and which, when introduced into the cell causes inhibition of expression by hybridizing with the mRNA and/or genomic sequences of an angiopoietin-like gene. Such oligomer probes are preferably modified oligomers that are resistant to endogenous nucleases, e.g. exonucleases and/or endonucleases, and are therefore stable *in vivo*. Molecules for use as antisense oligomers are selected from the group of, but not limited to phosphodi-  
 5 amide, phosphoramidate, phosphorothioate and methylphosphonate analogs of DNA (Froehler et al. (1988) Nucleic Acid Res. 16:4831-4839; Hyrup and Nielsen (1996) Bioorg. Med. Chem. 4:5-23, Sarin et al. (1988) Proc. Natl. Acad. Sci. USA. 85:7448-7451; Stein et al. (1988) Nucleic Acid Res. 16:3209-21; Summerton (1999) Biochim. Biophys. Acta. 1489: 141-158; Summerton and Weller (1997) Antisense Nucleic Acid Drug Dev. 7:187-195; Orum and Wengel (2001); Wahl-  
 10stedt et al., (2000). Additionally, general approaches to constructing oligomers useful in "antisense" therapy have been reviewed, in Letsinger et al. (1989) Proc. Natl. Acad. Sci. USA. 86:6553-6556; Summerton and Weller (1997) Antisense Nucleic Acid Drug Dev. 7:187-195; Froehler et al. (1988) Nucleic Acid Res. 16:4831-4839. With respect to antisense nucleotide sequences, oligomers derived from the translation initiation site, e.g., between the -10 and +10 regions of the angiopoietin-like nucleotide sequence of interest, are preferred.

**[0134]** Antisense approaches involve the design of oligonucleotides (either DNA or RNA, analogs, or derivatives thereof) that are complementary to angiopoietin-like mRNA. The antisense oligomers will bind to the angiopoietin-like mRNA transcripts and prevent translation. Absolute complementarity, although preferred, is not required. A sequence "complementary" to a portion of an RNA, as referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double-stranded antisense polynucleotide  
 20 sequences, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense polynucleotide sequence. Generally, the longer the hybridizing polynucleotide sequence, the more base mismatches with an RNA it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

**[0135]** Oligomers that are complementary to the 5' end of the message, e.g., the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences comple-  
 25 mentary to the 3' untranslated sequences of mRNAs have recently been shown to be effective at inhibiting translation of mRNAs as well (Wagner (1994) Nature. 372:333-335). Therefore, oligomers complementary to either the 5' or 3' untranslated, non-coding regions of an angiopoietin-like gene could be used in an antisense approach to inhibit trans-  
 30 lation of endogenous angiopoietin-like mRNA. Oligomers complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligomers complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the 5', 3' or coding region of angiopoietin-like mRNA, antisense oligomers should be at least six nucleotides in length, and are preferably oligomers ranging from 6 to about 50 nucleotides in length. In certain embodiments, the  
 35 oligomer is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides, or at least 50 nucleotides in length.

**[0136]** Regardless of the choice of the target sequence, it is preferred that *in vitro* studies are first performed to quantitate the ability of the antisense oligomer to inhibit gene expression. It is preferred that these studies utilize controls that distinguish between antisense gene inhibition and nonspecific biological effects of oligomers. It is also preferred that these studies compare levels of the target RNA or protein with that of an internal control RNA or protein. Additionally,  
 40 it is envisioned that results obtained using the antisense oligomer are compared with those obtained using control oligomers. It is preferred that the control oligonucleotide is of approximately the same length as the test oligomers and that the nucleotide sequence of the oligomers differs from the antisense sequence no more than is necessary to prevent specific hybridization to the target sequence.

**[0137]** The oligomers can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-  
 45 stranded or double-stranded. The oligomer can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligomer may include other appended groups such as peptides (e.g. for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see Letsinger et al. (1989) Proc. Natl. Acad. Sci. USA. 86:6553-6556; Lemaitre et al. (1987) Proc. Natl. Acad. Sci. USA. 84:648-652; PCT Publication No. WO 88/09810, published Dec. 15, 1988) or the blood-brain barrier (see PCT  
 50 Publication No. WO 89/10134, published Apr. 25, 1988), hybridization-triggered cleavage agents. (Van der Krol et al. (1988) Biotechniques 6:958-976) or intercalating agents (Zon (1988) Pharm. Res. 5:539-549). To this end, the oligomer may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

**[0138]** The antisense oligomer may comprise at least one modified base moiety which is selected from the group including but not limited to 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetyl-  
 55 cytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methyl-

guanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), -5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

**[0139]** The antisense oligomer may also comprise at least one modified sugar moiety selected from the group including but not limited to arabinose, 2-fluoroarabinose, xylulose, and hexose. In yet another embodiment, the antisense oligomer comprises at least one modified phosphate backbone selected from the group consisting of a phosphorothioate (Stein and Cohen (1989) In: Oligonucleotides: Antisense Inhibitors of Gene Expression. pp.97-117), a phosphorothioate, a phosphoramidothioate, a phosphoramidate (Froehler et al. (1988), Nucleic Acid. Res. 16:4831-4839), a phosphordiamidate, a methylphosphonate (Miller (1989) In: Oligonucleotides: Antisense Inhibitors of Gene Expression, pp.85-92), an alkyl phosphotriester (Miller (1989) In: Oligonucleotides: Antisense Inhibitors of Gene Expression. pp. 82-85), a formacetal or analog thereof, as well as  $\alpha$ -DNA (Rayner et al. (1989) In: Oligonucleotides: Antisense Inhibitors of Gene Expression. pp.119-136), 2'-O-methyl RNA (Shibahara et al. (1989), Nucleic Acid Res. 17:239-252), and morpholine backbone (Summerton and Weller (1997) Antisense Nucleic Acid Drug Dev. 7:187-195). Oligomers of the invention may be synthesized by standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligomers may be synthesized by the method of Stein et al. (1988) Nucleic Acid Res. 16:3209-3021), methylphosphonate oligomers can be prepared by use of controlled pore glass polymer supports (Sarin et al. (1988) Proc. Natl. Acad. Sci. USA. 85:7448-7451). Morpholino oligomers may be synthesised by the method of Summerton and Weller (1993) U. S. Patent Nos. 5,217,866 and 5,185,444, etc.

**[0140]** In specific embodiments, antisense molecules are delivered to cells that express angiopoietin-like proteins *in vivo*. A number of methods have been developed for delivering antisense DNA or RNA to cells; e.g., antisense molecules can be injected directly into the tissue site, or modified antisense molecules, designed to target the desired cells e.g., antisense linked to peptides or antibodies that specifically bind receptors or antigens expressed on the target cell surface can be administered systematically.

#### Polypeptides used in the present invention

**[0141]** One aspect of the invention pertains to the use of polypeptide sequences selected from the group consisting of SEQ ID Nos. 7 or 8 and equivalents thereof for the treatment of blood related disorders or defects in vasculature. The term equivalent is understood to include functionally equivalent polypeptides having an activity of an angiopoietin-like protein such as described herein.

**[0142]** Polypeptides, used according to the present invention, are at least 55% identical or similar to the amino acid sequences of SEQ ID Nos. 7 or 8. Polypeptides which are at least about 70%, and even more preferably at least about 80%, 85%, 90%, 95%, or 98% identical or similar to the amino acid sequences represented in SEQ ID Nos. 7 or 8 are also within the scope of the invention.

**[0143]** In some preferred embodiments of the invention polypeptides are used which are at least about 70%, preferably at least about 80%, and most preferably at least about 90% identical or similar to a portion of at least about 10 consecutive amino acids of the amino acid sequence set forth in SEQ ID Nos. 7 or 8. In one embodiment, the portion corresponds to about amino acid 1 to about amino acid 493 of SEQ ID Nos. 7 or 8.

**[0144]** In a particularly preferred embodiment, polypeptides have an overall amino acid sequence homology or identity of at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99% with the amino acid sequences shown in SEQ ID Nos. 7 or 8 are used according to the present invention. The polypeptides include angiopoietin-like proteins from any species. Preferred polypeptides are vertebrate angiopoietin-like proteins. More preferred polypeptides are those of primate angiopoietin-like proteins including mammalian angiopoietin-like proteins, e.g., human angiopoietin-like proteins. Other polypeptides used in the invention include fish, amphibian, avian, equine, canine, feline, bovine or porcine angiopoietin-like proteins.

**[0145]** In a preferred embodiment, the polypeptide includes a polypeptide sequence corresponding to all or a portion of the amino acid sequence of SEQ ID Nos. 7 or 8, e.g. at least 2, 5, 10, 25, 50, 100, 150 or 200 amino acids of that region. Yet other preferred polypeptides comprise at least about 3, at least about 5, at least about 10, at least about 15, at least about 20, or at least about 25 consecutive amino acids from about amino acid 1 to about amino acid 493 set forth in SEQ ID Nos. 7 or 8.

**[0146]** In another preferred embodiment of the invention, the polypeptides comprise a signal sequence, coiled-coil domain and a fibrinogen-like domain, such as, angiopoietin-like proteins having SEQ ID Nos. 7 or 8. Other preferred polypeptides comprise a fibrinogen-like domain. Yet other preferred polypeptides comprise at least of one of the conserved motifs in the fibrinogen domain, e.g.  $\text{Ca}^{++}$ -binding motif. In another preferred embodiment, the polypeptides correspond to angiopoietin-like proteins essentially lacking the fibrinogen-like domain. Other preferred polypeptides

comprise a conserved coiled-coil domain of angiopoietins.

**[0147]** Polypeptides selected from the above-described amino acid sequences, can be soluble. Preferred soluble peptides comprise at least a portion of the fibrinogen-like domain of an angiopoietin-like protein. Even more preferred, soluble polypeptides comprise a portion of the fibrinogen-like domain, including the  $\text{Ca}^{++}$ -binding motif of an angiopoietin-like protein consisting of the amino acid sequence SEQ ID No. 9. Furthermore, soluble polypeptides that comprise a portion of the fibrinogen-like domain, the  $\text{Ca}^{++}$ -binding motif, and the fibrinogen beta and gamma chains in the C-terminal domain consisting of the amino acid SEQ ID No. 10 are envisioned by the present invention. Moreover, such preferred soluble polypeptides may comprise a portion of the fibrinogen-like domain, the  $\text{Ca}^{++}$ -binding motif, the fibrinogen beta and gamma chains in the C-terminal domain and a coiled-coil domain. Such soluble polypeptides may in addition comprise a signal sequence consisting of SEQ ID No. 11.

**[0148]** In yet other preferred embodiments, the polypeptides used in the invention comprise at least one domain of an angiopoietin-like protein selected from the group consisting of: a fibrinogen-like domain, an  $\text{Ca}^{++}$ -binding motif consisting of the amino acid sequence SEQ ID No. 9, a fibrinogen beta and gamma chains in the C-terminal domain consisting of the amino acid sequence of SEQ ID No. 10, a coiled-coil domain, and/or a signal sequence consisting of the amino acid sequence of SEQ ID No. 11, and all phosphorylation sites required for the polypeptides to be activated (Figure 3). The polypeptides can comprise several of these domains from an angiopoietin-like protein. Alternatively, a polypeptide can be a chimeric protein, i.e., comprising several of these conserved domains, at least some of which are derived from a protein other than an angiopoietin-like protein.

**[0149]** Polypeptides and fragment polypeptides that are capable of exhibiting biological activity are also used in the present invention. Fragments of the protein may be in linear form or they may be cyclized using known methods, for example, as described in Saragovi et al. (1992) Biotechnology. 10:773-778.

**[0150]** Angiopoietin-like polypeptides as fusion proteins can be used in this invention. Such fusion proteins can comprise at least a portion of the kinase domain of an angiopoietin-like polypeptide. Polynucleotide sequences encoding such fusion proteins can be prepared as described in U.S. Patent No. 5,434,131.

Polynucleotides encoding polypeptides used in the present invention

**[0151]** Another aspect of the invention pertains to the use of isolated polynucleotide sequences comprising nucleotide sequences encoding angiopoietin-like polypeptides, and/or equivalents thereof. The term equivalent is understood to include nucleotide sequences encoding functionally equivalent angiopoietin-like polypeptides or functionally equivalent peptides having an activity of an angiopoietin-like protein such as described herein. Equivalent nucleotide sequences will include sequences that differ by one or more nucleotide substitutions, additions or deletions, such as allelic variants; and will, therefore, include sequences that differ from the polynucleotide sequences of the angiopoietin-like genes shown in any of SEQ ID Nos. 1, 2, 3, or 4 due to the degeneracy of the genetic code.

**[0152]** Polynucleotides encoding polypeptide sequences set forth in the foregoing section "Polypeptides used in the present invention" are all envisioned for use in the various embodiments of the present invention.

**[0153]** Preferred polynucleotides have a sequence at least about 75% homologous and more preferably at least about 80% and even more preferably at least about 85% homologous with a polynucleotide sequence of an angiopoietin-like gene, such as human angiopoietin-like 1 gene, e.g., such as a sequence shown in one of SEQ ID Nos. 2 or 3. Polynucleotide sequences at least about 90%, more preferably at least about 95%, and most preferably at least about 98-99% homologous with a polynucleotide sequence represented in one of SEQ ID Nos. 2 or 3 are of course also within the scope of the invention. In preferred embodiments, the polynucleotide is a human angiopoietin-like gene and, in particularly preferred embodiments, includes all or a portion of the nucleotide sequence corresponding to the coding region of one of SEQ ID Nos. 2 or 3.

**[0154]** Furthermore, polynucleotide sequences having sequences that differ from the polynucleotide sequences shown in one of SEQ ID Nos. 2 or 3 due to degeneracy of the genetic code, can be used in the invention. Such polynucleotides encode functionally equivalent peptides (i.e., a peptide having a biological activity of an angiopoietin-like polypeptide) but differ in sequence from the sequence shown in the sequence listing due to degeneracy in the genetic code. For example, a number of amino acids are designated by more than one triplet. Codons that specify the same amino acid, or synonyms (e.g., GCU, GCC, GCA, and GCG encode alanine), may result in "silent" mutations that do not affect the amino acid sequence of an angiopoietin-like polypeptide. However, it is expected that DNA sequence polymorphisms that do lead to changes in the amino acid sequences of the subject angiopoietin-like polypeptides will exist. One skilled in the art will appreciate that these variations in one or more nucleotides (e.g. up to about 3-5% of the nucleotides) of the polynucleotide encoding polypeptides having a biological activity of an angiopoietin-like polypeptide may exist among individuals of a given species due to natural allelic variation.

**[0155]** Angiopoietin-like protein encoding polynucleotide sequences can be obtained from mRNA present in any of a number of eukaryotic cells. It should also be possible to obtain polynucleotide sequences encoding angiopoietin-like polypeptides used in the present invention from genomic DNA from both adults and embryos. For example, screening



cDNA or genomic libraries (Benton et al. (1977) Science. 196:180-182). Purification of such polynucleotides from suitable cell lines or libraries, specific for a particular tissue (e.g. ATCC No. CCL-246, a cell line derived from human bone marrow, specific for myelogenous leukemia). A cDNA encoding an angiopoietin-like protein can be obtained by isolating total mRNA from a cell, e.g. a vertebrate cell, a mammalian cell, or a human cell, including embryonic cells, in accordance with protocols described within Current Protocols in Molecular Biology (1989) 4.5.1-4.5.3. The gene encoding an angiopoietin-like protein can also be cloned using established polymerase chain reaction techniques (PCR) in accordance with the nucleotide sequence information provided by the invention (Saiki et al. (1988) Science. 239:487-491; and Schaefer (1995) Analytical Biochem. 227:255-273). The polynucleotide sequence of the invention can be DNA or RNA. A preferred polynucleotide sequence is a cDNA represented by a sequence selected from the group consisting of SEQ ID Nos. 2 or 3.

#### Probes and Primers

**[0156]** Moreover, the polynucleotide sequences determined from human angiopoietin-like genes will further allow for the generation of probes and primers designed for use in identifying and/or cloning angiopoietin-like homologs in other cell types, e.g. from other tissues, as well as angiopoietin-like homologs from other mammalian organisms. Probes and primers can also be used to determine the identity of an angiopoietin-like allele and/or the presence or absence of one or more mutations in an angiopoietin-like gene of a subject. In a preferred embodiment, a probe or primer used in the invention can be used to determine whether a subject has or is at risk of developing a disease or condition associated with a specific angiopoietin-like allele, such as an allele carrying a mutation.

**[0157]** Preferred polynucleotide sequences for use as a probe, according to the methods of the invention, include polynucleotide sequences comprising a nucleotide sequence having at least about 6, preferably at least about 9, more preferably at least about 12 and even more preferably at least about 15 consecutive nucleotides from SEQ ID Nos. 2 or 3 or from a portion thereof. In a preferred embodiment, the portion corresponds to about nucleotide 1 to about nucleotide 2066 of SEQ ID Nos. 2 or 3. Alternatively a portion can be a nucleotide sequence encoding a conserved motif of human angiopoietin-like protein, e.g., coiled-coil or fibrinogen-like domain. Alternatively, the portion can be a nucleotide sequence located between polynucleotide sequences encoding conserved motifs of human angiopoietin-like protein.

**[0158]** The invention further pertains to polynucleotide sequence molecules for use as probes/primer or antisense molecules (i.e. non-coding polynucleotide sequence molecules), which can comprise at least about 6, 12, 20, 30, 50, 100, 125, 150 or 200 nucleotides or base pairs. Yet other preferred polynucleotide sequences comprise at least about 300, at least about 350, at least about 400, at least about 450, at least about 500, or at least about 600 nucleotides of SEQ ID Nos. 2 or 3. In some embodiments, the polynucleotide sequences of the invention correspond to the 5' portion of the polynucleotide sequences shown SEQ ID Nos. 2 or 3. For example, a polynucleotide sequence used in the invention can correspond to a portion of about nucleotide 1 to about nucleotide 2066 of the polynucleotide sequences shown in SEQ ID Nos. 2 or 3.

**[0159]** Also used in the invention are polynucleotide sequences that are capable of hybridizing to an angiopoietin-like polypeptide sequence, and in particular, to those shown in SEQ ID Nos. 2 or 3, and fragments thereof under various conditions of stringency. Conditions that promote hybridization of polynucleotide sequences are known to those skilled in the art (see Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989) 6.3.1-6.3.6, Jowett (2001) Methods. 23:345-358). For example, stringent salt concentration will ordinarily be less than about 750mM NaCl and 75mM trisodium citrate. High stringency hybridization can be obtained in the presence of at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least 45 degree Celsius, more preferably of at least 55 degree Celsius, and most preferably of at least about 65 degree Celsius. Varying additional parameters, such as hybridization time, the concentration of detergent, e.g., Tween 20, and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency can be accomplished by combining these various conditions as needed. In a preferred embodiment, hybridization will occur at 45 degree Celsius in 750mM NaCl, 75mM trisodium citrate, and 0.1% Tween 20, 50% formamide, 500µg/ml yeast RNA and 50µg/ml heparin. In a more preferred embodiment hybridization will occur at 55 degree Celsius in 750mM NaCl, 75mM trisodium citrate, and 0.1% Tween 20, 50% formamide, 500µg/ml yeast RNA and 50µg/ml heparin. In a more preferred embodiment hybridization will occur at 65 degree Celsius in 750mM NaCl, 75mM trisodium citrate, and 0.1% Tween 20, 50% formamide, 500µg/ml yeast RNA and 50µg/ml heparin. Useful variation on these conditions will be readily apparent to those skilled in the art. The stringency of the wash steps, which follow hybridization, is defined by the salt concentration and by temperature. For example, stringent salt concentrations for the wash steps will preferably be less than about 30mM NaCl and 3mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include temperatures of at least 45 degree Celsius, more preferably at least 55 degree Celsius, and most preferably at 65 degree Celsius. In a preferred embodiment, wash steps will occur at 65 degree Celsius in 30mM NaCl, 3mM trisodium citrate, and 0.1% Tween-20. Additional variation on these conditions will be readily apparent to those skilled in the art.



**[0160]** In a preferred embodiment, the present invention also provides a probe/primer comprising a substantially purified oligonucleotide, which oligonucleotide comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12 (e.g. 12), preferably about 25 (e.g. 25), more preferably about 40, 50 or 75 (e.g. 40, 50, or 75 respectively) consecutive nucleotides of sense or antisense sequence of the nucleotide sequence selected from the group consisting of SEQ ID Nos. 2 or 3, or naturally occurring mutants thereof. For instance, primers based on the polynucleotide sequence represented in SEQ ID Nos. 2 or 3 can be used in PCR reactions to clone angiopoietin-like homologs, e.g. specific angiopoietin-like alleles. Such primers are preferably selected in a region that does not share significant homology to other genes, e.g., other fibrinogen domain containing genes. Likewise, probes based on the subject angiopoietin-like sequences can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In preferred embodiments, the probe further comprises a label group attached thereto and able to be detected, e.g. the label group is selected from amongst radioisotopes, fluorescent compounds, enzymes, and enzyme co-factors.

**[0161]** Such probes can also be used as a part of a diagnostic test kit for identifying cells or tissue which mis-express an angiopoietin-like protein, such as by measuring a level of an angiopoietin-like protein encoding polynucleotide sequence in a sample of cells from a patient; e.g. detecting angiopoietin-like mRNA levels or determining whether a genomic angiopoietin-like gene has been mutated or deleted. Briefly, nucleotide probes can be generated from the subject angiopoietin-like genes that facilitate histological screening of intact tissue and tissue samples for the presence (or absence) of angiopoietin-like protein encoding transcripts. Similar to the diagnostic uses of anti-angiopoietin-like antibodies, the use of probes directed to angiopoietin-like messages, or to genomic angiopoietin-like sequences, can be used for both predictive and therapeutic evaluation of allelic mutations which might be manifested in, for example, blood disorders (e.g. anemia) (e.g. unwanted cell growth) or abnormal differentiation of tissue. Used in conjunction with immunoassays as described herein, the oligonucleotide probes can help facilitate the determination of the molecular basis for a developmental disorder which may involve some abnormality associated with expression (or lack thereof) of an angiopoietin-like protein. For instance, variation in polypeptide synthesis can be differentiated from a mutation in a coding sequence. Also within the scope of the invention are kits for determining whether a subject is at risk of developing a disease or condition caused, or contributed, by an aberrant angiopoietin-like bioactivity and/or which is associated with one or more specific angiopoietin-like alleles. In a preferred embodiment, the kit can be used for determining whether a subject is at risk of developing a blood disorder, e.g. anemia or leukemia, or defects in vasculature.

### Vectors

**[0162]** Expression vectors containing a polynucleotide sequences encoding an angiopoietin-like polypeptide and being operably linked to at least one transcriptional regulatory sequence may be used for practicing the present invention. Regulatory sequences are recognized by those skilled in the art, and are specifically selected to direct expression of the subject angiopoietin-like proteins. Such regulatory sequences are described in Rodriguez and Chamberlain (1982) eds. In: Promoters: Structure and function. NY. Praeger; Khoury and Gruss (1983) Cell. 33:313-314. In one embodiment, the expression vector includes a recombinant gene encoding a peptide having an agonistic activity of a subject angiopoietin-like polypeptide, or alternatively, encoding a peptide which is an antagonistic form of the angiopoietin-like protein. Such expression vectors can be used to transfect cells and thereby produce polypeptides, including fusion proteins, encoded by polynucleotide sequences as described herein. Moreover, the gene constructs can be used as a part of a gene therapy protocol to deliver polynucleotide sequences encoding either an agonistic or antagonistic form of an angiopoietin-like protein. Thus, another aspect of the invention features expression vectors for *in vivo*, *in vitro*, or *ex vivo* transfection and expression of an angiopoietin-like polypeptide in particular cell types so as to reconstitute the function of, or alternatively, abrogate the function of angiopoietin-like activity (e.g. stimulation of vascular remodeling or activating differentiation of hematopoietic progenitor cells into blood cells). This could be desirable, for example, when the naturally occurring form of the protein is mis-expressed; or to deliver a form of the protein which alters differentiation of tissue (e.g. inhibiting or altering differentiation of hematopoietic stem cells).

**[0163]** In addition to viral transfer methods, non-viral methods can also be employed to cause expression of angiopoietin-like polypeptides in the tissue of an animal. Most nonviral methods of gene transfer rely on normal mechanisms used by mammalian cells for the uptake and intracellular transport of macromolecules. In preferred embodiments, non-viral targeting means rely on endocytic pathways for the uptake of an angiopoietin-like polypeptide gene by the targeted cell. Exemplary targeting means of this type include liposomal derived systems, poly-lysine conjugates, and artificial viral envelopes.

**[0164]** The present invention is further illustrated by the following examples, which should not be construed as limiting in any way. The contents of all cited references (including literature references, issued patents, published patent applications), as cited throughout this application, are hereby expressly incorporated by reference. The practice of the present invention will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are known to those skilled in the

art. Such techniques are explained fully in the literature.

#### Example 1. Homology analyses of Angiopoietin Y1

**[0165]** A search for angiopoietin family members in the zebrafish expressed sequence tag (zfEST) database of the National Center for Biotechnology Information (NCBI) revealed the existence of a partial cDNA with Accession Number AW174181 (Sugano et al., 1999). The zfEST was analyzed using the NCBI ORF Finder program in order to identify the longest open reading frame of the partial cDNA. The resulting nucleotide sequence was then translated into a polypeptide sequence (ORF Finder program) and a BLASTP search (Altschul et al., 1997) to identify homologous protein sequences was performed. Results of the BLASTP analysis showed that the gene encoded a polypeptide possessing high homology to the angiopoietin-like (ANGPTL) family of proteins (see Figures 1 and 2). Alignment of the zebrafish translated-EST for angiopoietin Y1 (ANGY1) with the amino acid sequences for human and mouse ANGPTL1 (ANGY1), ANGPTL2 (or angiopoietin-related; ARP2), ANGPTL3, ANGPTL4, ANGPTL5 (ARP5) and ANGPTLF (angiopoietin-like factor) reveals that the isolated zebrafish EST is closely related to a subset of the ANGPTL family, mainly ANGPTL1 and ANGPTL2 (based on results of a ClustalW computational analysis). In particular, the isolated zebrafish EST holds the highest degree of homology to human ANGPTL1, indicating that this partial nucleotide sequence is most likely the zebrafish homologue. Furthermore, alignment of amino acid sequences for angiopoietin-like proteins in human, mouse and zebrafish also revealed striking sequence similarities across species, particularly within several functional domains. Both human and mouse ANGPTL1 and ANGPTL2 contain a secretory signal sequence (SEQ ID NO: 11) at the N-terminus (Figure 3, underlined), which is also conserved in zebrafish. An N-terminal coiled-coil domain (Figure 3, marked by small solid arrows), similar to the coiled-coil domains found in most angiopoietins (ANG), encompasses one third of the polypeptide. Coiled-coil domains of angiopoietins have been shown to mediate oligomerization of angiopoietin molecules, *in vitro* (Propocio et al., 1999). Based on amino acid alignments of ANG1, ANG2, ANGPTL1 and ANGPTL2, this coiled-coil domain is well conserved within the angiopoietin family, suggesting a similar role in ANGPTL molecules (Propocio et al., 1999). This coiled-coil is followed by a short sequence of amino acids that links this domain with that of the fibrinogen-like domain; hence linker (Conklin et al., 1999). The fibrinogen-like domain, (Figure 3, marked by large, outlined arrows) is not only highly conserved across species, but also across the angiopoietin family of proteins as the receptor-ligand binding domain where it functions as a mediator of agonistic (ANG1) and antagonistic (ANG2) effects of these ligand (Xu et al., 1996; Propocio et al., 1999). Furthermore, this domain contains a Ca<sup>++</sup>-binding motif (SEQ ID NO: 9) (Figure 3, underlined) also present in most members of the angiopoietin-like family of proteins (Conklin et al., 1999), as well as a signature 'fibrinogen beta and gamma chains' domain at the C-terminal end of the molecule (SEQ ID NO: 10) (Figure 3, bold). Moreover, several conserved cysteine residues can be found within the aligned angiopoietin-like proteins (Figure 3, underlined astericks). These cysteine residues form disulfide bridges similar to those found in angiopoietin molecules, indicating that the secondary structure of angiopoietin-like molecules is comparable to that of the angiopoietin molecules. Interestingly, ANGPTL1 and ANGPTL2 contain an extra cysteine residue within the N-terminal portion of the protein which is not found in other members of the angiopoietin-like family of proteins. This amino acid residue is conserved in angiopoietin 1 (ANG1) and angiopoietin 2 (ANG2) (Kim et al., 1999b).

#### Example 2. Inhibition of the zebrafish angiopoietin-like 1 precursor (zfANGPTL1) transcript via morpholino antisense mediated knockdown

**[0166]** In order to determine whether ANGPTL1 plays a role in hematopoiesis/erythropoiesis, an antisense oligomer of the morpholino structural type was designed to target the region flanking the initiation codon of the zebrafish ANGPTL1 transcript (nucleotides 2-26 of zfEST, with ATG starting at nucleotide 5), resulting in inhibition of protein synthesis (gene 'knock-down'). The said zfANGPTL1-specific morpholino oligomer was synthesized with the following nucleotide base sequence:

**5'-ACATCCAGACCTGAGGACACATAGC-3' (SEQ ID NO: 5).**

**[0167]** Approximately 0.25 nl to 1.0 nl of said morpholino oligomer at 1:10 and 1:50 dilutions of a 2mM stock solution, were microinjected into single-cell stage zebrafish embryos using back-filled fine borosilicate glass capillary needles (Egger, 2000; Nasevicius and Egger, 2000). Live morphological analysis of treated embryos at 1 day post-fertilization (dpf) revealed no obvious morphological defects.

**[0168]** Since hematopoiesis in the zebrafish begins before 1 dpf, o-dianisidine staining of hemoglobin was performed with embryos injected with 40µM, 100µM and 200µM of said morpholino oligomer, after 2dpf and 3dpf. O-dianisidine staining (Detrich III, et al., 1995) of same treated zebrafish embryos at 2 dpf revealed a dose-dependent effect of said

morpholino oligomer on hemoglobin staining (Figure 11). At 40µM, the overall hemoglobin staining is unaffected and all embryos appear normal. However, at both 100 and 200µM, there is a complete loss of hemoglobin staining, suggesting that zfANGPTL1 plays a role in either hematopoiesis or erythropoiesis. Wild-type (wt) embryos showed no signs of alterations in hemoglobin staining, indicating that the loss of hemoglobin staining in embryos injected with said morpholino oligomer was specific to the gene 'knock-down' of zfANGPTL1 (Figure 11). Control embryos injected with a morpholino oligomer designed specifically against uroporphyrinogen decarboxylase (UROD) revealed expected results (data not shown). Inhibition of protein synthesis of UROD, a decarboxylase important in the heme synthesis pathway, results in the inability of the erythrocyte/reticulocyte to convert uroporphyrinogen→coproporphyrinogen, thereby causing an accumulation of uroporphyrinogen within the cell cytoplasm, and ultimately to an absence of heme.

[0169] Interestingly, o-dianisidine staining analysis of treated embryos after 3dpf revealed similar results (Figure 11). Injection of said morpholino oligomer at 40µM did not result in a reduction/loss of hemoglobin staining, as expected. However at both 100µM and 200µM concentrations of said morpholino oligomer, staining of hemoglobin appears to be reduced, unlike the complete loss of hemoglobin staining found after 2dpf. Wild-type (wt) embryos showed no signs of changes in hemoglobin staining, indicating that the loss of hemoglobin staining in embryos injected with said morpholino oligomer is specific to the gene 'knock-down' of zfANGPTL1 (Figure 11). Embryos injected with a morpholino oligomer designed specifically against UROD revealed expected results (data not shown).

### Example 3. Whole mount in situ hybridization analysis

[0170] In order to determine whether loss of hemoglobin staining in the initial studies is the direct result of an alteration in hematopoiesis, erythropoiesis and/or angiogenesis due to the ANGPTL1 gene knock-down, wholemount *in situ* hybridisation analysis (Hauptmann, 1999) was performed on treated embryos using tissue-specific genetic markers for blood progenitors and early vasculature (Thompson et al., 1998; Brown et al., 2000; Amatruda and Zon, 1999; Long et al., 1997). Analysis of treated embryos at 18 somite stage and 1dpf for zebrafish GATA-1 marker expression for early erythroid progenitors revealed that ANGPTL1 may not play a role in early hematopoiesis, since early erythroid progenitors are not directly affected by ANGPTL1 gene 'knock-down' (Figure 2a). Furthermore, expression analysis of angiogenesis markers flk-1, fli-1 and flk4, revealed some defects in expression patterns of treated embryos. flk-1 and fli-1 marker (Figure 12c and d) expression of treated embryos is slightly reduced at a morpholino concentration of 100µM, whereas at 40µM and 200µM there is no significant reduction in expression of these two markers. flt-4 marker expression showed a dose-dependent effect in treated embryos at the 18-somite stage; at 40µM the expression pattern of both markers was reduced in a small number of embryos, whereas at 100µM and 200µM the number of embryos with reduced expression increased (Figure 12b), indicating that ANGPTL1 plays a role in angiogenesis. Interestingly, at 24 hpf there is no significant reduction of Flt-4 marker expression in treated embryos. Furthermore, the expression pattern of lmo-1, a marker for primitive hematopoiesis, is unaffected by the 'knock-down' of ANGPTL1 (Figure 12e). that the above results suggest that the loss of hemoglobin staining observed in ANGPTL1 'knockdown' zebrafish embryos clearly demonstrates the role of ANGPTL1 in later, rather than early, stages of erythropoiesis.

## SEQUENCE LISTING

<110> Mermaid Pharmaceuticals GmbH

<120> Materials and Methods of Using Angiopoietin-like 1 and  
Angiopoietin-like 2 Genes for Altering Clinical Status

<130> 260-34 EP

<150> US 60/335,362

<151> 2001-10-31

<160> 11

<170> PatentIn Ver. 2.1

<210> 1

<211> 444

<212> DNA

<213> Danio rerio

<400> 1

tgctatgtgt	cctcaggtct	ggatgttctg	ctggttcctc	tgtgtgctgt	ggtgtgtgcg	60
gatcagcagt	tcctccagcc	ggcagcgcag	gcagatggag	gctccagacc	cgcagcggca	120
gcagcagtgc	tcctacacct	tcttaatccc	ggagcagaag	atcacggggc	ctatctgtgc	180
cagccggggg	ccctcgtctg	cggaccccca	gcgcgtgacg	cggacggaca	tgtctgagct	240
gcggcagacc	ctgagccggc	agcggcgagg	gatggagacg	ctgcggatga	tgggtggatgt	300
ggacggagac	atggtgcagg	agatgaagct	gctgcgtaag	gagtcgcgga	acatgaactc	360
gcgggtcact	cagctctaca	tgcagctgct	gcacgaggtc	ctgcgcaagc	gcgactcctc	420
tctggagctg	gcggcgctgc	agaa				444

<210> 2

<211> 2066

<212> DNA

<213> Homo sapiens

<400> 2

atttctccat	gtggcagaca	gagcaaagcc	acaacgcttt	ctctgctgga	ttaaagacgg	60
cccacagacc	agaacttcca	ctatactact	taaaattaca	taggtggctt	gtcaaattca	120
attgattagt	attgtaaaag	gaaaaagaag	ttccttctta	cagcttggat	tcaacgggtcc	180
aaaacaaaaa	tgcagctgcc	attaaagtca	cagatgaaca	aacttctaca	ctgattttta	240
aatcaagaa	taagggcagc	aagtttcttg	attcactgaa	tcaacagaca	caaaaagctg	300
gcaatatagc	aactatgaag	agaaaagcta	ctaataaaat	taacccaacg	catagaagac	360
ttttttttct	cttctaaaaa	caactaagta	aagacttaaa	tttaaacaca	tcatttttaca	420
acctcatttc	aaaatgaaga	cttttacctg	gaccctaggt	gtgctattct	tcctactagt	480
ggacactgga	cattgcagag	gtggacaatt	caaaattaaa	aaaataaacc	agagaagata	540
ccctcgtgcc	acagatggta	aagaggaagc	aaagaaatgt	gcatacacat	tcctgggtacc	600
tgaacaaaga	ataacagggc	caatctgtgt	caacaccaag	gggcaagatg	caagtaccat	660
taaagacatg	atcaccagga	tggaccttga	aaacctgaag	gatgtgctct	ccaggcagaa	720
gcgggagata	gatgttctgc	aactgggtgt	ggatgtagat	ggaaacattg	tgaatgaggt	780
aaagctgctg	agaaaggaaa	gccgtaacat	gaactctcgt	gttactcaac	tctatatgca	840
attattacat	gagattatcc	gtaagaggga	taattcactt	gaactttccc	aactggaaaa	900
caaaatcctc	aatgtcacca	cagaaatgtt	gaagatggca	acaagataca	gggaactaga	960
ggtgaaatac	gcttccttga	ctgatcttgt	caataaccaa	tctgtgatga	tcactttgtt	1020
ggaagaacag	tgtttgagga	tattttcccg	acaagacacc	catgtgtctc	ccccacttgt	1080
ccaggtggtg	ccacaacata	ttcctaacag	ccaacagtat	actcctggtc	tgctgggagg	1140
taacgagatt	cagagggatc	caggttatcc	cagagattta	atgccaccac	ctgatctggc	1200
aacttctccc	acaaaaagcc	ctttcaagat	accaccggta	actttcatca	atgaaggacc	1260
attcaaagac	tgtcagcaag	caaaagaagc	tgggcattcg	gtcagtggga	tttatatgat	1320
taaacctgaa	aacagcaatg	gaccaatgca	gttatggtgt	gaaaacagtt	tggaccctgg	1380



EP 1 308 511 A1

	gggttggact	gttattcaga	aaagaacaga	cggctctgtc	aacttcttca	gaaattggga	1440
	aaattataag	aaagggtttg	gaaacattga	cggagaatac	tggttggac	tggaataat	1500
	ctatatgctt	agcaatcaag	ataattacaa	gttattgatt	gaattagaag	actggagtga	1560
5	taaaaaagtc	tatgcagaat	acagcagctt	tcgtctggaa	cctgaaagtg	aattctatag	1620
	actgcgcctg	ggaacttacc	agggaaatgc	aggggattct	atgatgtggc	ataatggtaa	1680
	acaattcacc	acactggaca	gagataaaga	tatgtatgca	ggaaactgcg	cccactttca	1740
	taaaggaggc	tggtggtaca	atgcctgtgc	acattctaac	ctaaatggag	tatggtacag	1800
	aggaggccat	tacagaagca	agcaccaaga	tggaattttc	tgggccgaat	acagaggcgg	1860
	gtcatactcc	ttaagagcag	ttcagatgat	gatcaagcct	attgactgaa	gagagacact	1920
10	cgccaattta	aatgacacag	aactttgtac	ttttcagctc	ttaaaaatgt	aaatgttaca	1980
	tgtatattac	ttggcacaat	ttatttctac	acagaaagtt	tttaaaatga	attttaccgt	2040
	aactataaaa	gggaacctat	aatgt				2066

15  
 <210> 3  
 <211> 1518  
 <212> DNA  
 <213> Homo sapiens

	<400> 3						
20	aaccaccatt	ttgcaaggac	catgaggcca	ctgtgctga	catgctggtg	gctcggactg	60
	ctggctgcca	tgaggagctgt	tgcaggccag	gaggacggtt	ttgagggcac	tgaggagggc	120
	tcgccaagag	agttcattta	cctaaacagg	tacaagcggg	cgggcgagtc	ccaggacaag	180
	tgcacctaca	ccttcattgt	gccccagcag	cgggtcacgg	gtgccatctg	cgtcaactcc	240
	aaggagcctg	aggtgcttct	ggagaaccga	gtgcataagc	aggagctaga	gctgctcaac	300
	aatgagctgc	tcaagcagaa	gcggcagatc	gagacactgc	agcagctggt	ggagggtggac	360
25	ggcggcattg	tgagcgaggt	gaagctgctg	cgcaaggaga	gccgcaacat	gaactcgcgg	420
	gtcacgcagc	tctacatgca	gtcctgcac	gagatcatcc	gcaagcggga	caacgcgttg	480
	gagctctccc	agctggagaa	caggatcctg	aaccagacag	ccgacatgct	gcagctggcc	540
	agcaagtaca	aggacctgga	gcacaagtac	cagcacctgg	ccacactggc	ccacaaccaa	600
	tcagagatca	tcgcgcagct	tgaggagcac	tgccagaggg	tgccctcggc	caggcccgtc	660
	ccccagccac	cccccgctgc	cccgccccgg	gtctaccaac	caccaccta	caaccgcac	720
30	atcaaccaga	tctctacca	cgagatccag	agtgaccaga	acctgaaggt	gctgccaccc	780
	cctctgcccc	ctatgcccac	tctcaccagc	ctcccatctt	ccaccgacaa	gccgtcgggc	840
	ccatggagag	actgcctgca	ggccttgag	gatggccacg	acaccagctc	catctacctg	900
	gtgaagccgg	agaacaccaa	ccgcctcatg	caggtgtggt	gcgaccagag	acacgacccc	960
	gggggctgga	ccgtcatcca	gagacgcctg	gatggctctg	ttaacttctt	caggaactgg	1020
35	gagacgtaca	agcaagggtt	tgggaacatt	gatggcgaat	actggctggg	cctggagaac	1080
	atttactggc	tgacgaacca	aggcaactac	aaactcctgg	tgaccatgga	ggactggtcc	1140
	ggccgcaaag	tctttgcaga	atacgccagt	ttccgcctgg	aacctgagag	cgagtattat	1200
	aagctgcggc	tggggcgcta	ccatggcaat	gcgggtgact	cctttacatg	gcacaacggc	1260
	aagcagttca	ccaccctgga	cagagatcat	gatgtctaca	caggaaactg	tgcccactac	1320
	cagaagggag	gctggtggtg	taacgcctgt	gcccactcca	acctcaacgg	ggtctggtac	1380
40	cgcgggggcc	attaccggag	ccgctaccag	gacggagtct	actgggctga	gttccgagga	1440
	ggctcttact	cactcaagaa	agtggatgat	atgatccgac	cgaaccccaa	caccttccac	1500
	taagccagct	ccccctcc					1518

45  
 <210> 4  
 <211> 1518  
 <212> DNA  
 <213> Mus musculus

	<400> 4						
50	aaccagcaca	ctgcaaggac	catgaggcca	ctgtgtatga	cctactggtg	gcttggactg	60
	ctggccacgg	tcggagctgc	tacaggccca	gaggctgacg	ttgagggcac	agaggatggt	120
	tcacagagag	agtacattta	cctcaacagg	tacaagcggg	caggtgagtc	ccccgacaag	180
	tgcacctaca	ctttcattgt	gccccagcag	cgggtcacag	gtgccatttg	tgtcaactcc	240
	aaggagcctg	aggtgcacct	ggagaaccgt	gtgcacaagc	aggagctgga	gctgctcaac	300
	aatgagctgc	ttaagcagaa	gcggcagatc	gagacgctgc	agcagctggt	agaggtagac	360
55	ggaggcatcg	tgagcgaggt	gaagctgctg	cgcaaggaga	gccgcaacat	gaactcgaga	420

# EP 1 308 511 A1

5 gtcacgcagc tgtacatgca acttctacat gagatcattc gaaagcgaga caatgcgctg 480  
 gagctctccc agctggagaa caggatcctg aaccagacag ctgacatgct gcagctggct 540  
 agcaagtaca aggacctgga gcacaagttc cagcacctgg ctatgctggc acacaaccaa 600  
 tcagaggtca ttgctcagct cgaagagcac tgccaacgcg tacctgcagc caggcctatg 660  
 cccagccac cccagcagc tccacctcgg gtctaccaac caccaccta caaccgcatc 720  
 atcaaccaga tttccaccaa tgagatccag agtgaccaga atctgaaggt gctgccgccc 780  
 tccttgccca ccatgcctgc ccttaccagt ctcccatctt ccaactgataa gccatcaggt 840  
 ccatggagag actgcctgca agccctggaa gatggtcaca gcaccagctc catctacctg 900  
 10 gtgaagcctg agaataccaa ccgcctgatg caggtgtggt gtgaccagag acatgaccct 960  
 ggaggttgga ctgtcatcca gagacgcctg gatggctctg tcaacttctt caggaactgg 1020  
 gagacctata agcaagggtt tgggaacatc gatggtgagt actggctggg cctggagAAC 1080  
 atctactggc tgacgaacca aggcaactac aaactgctgg taaccatgga ggactggtct 1140  
 ggccgtaaag tctttgctga gtatgccagt ttccgactgg agccagaaag cgagtactat 1200  
 aagctgcggc tggggcgcta tcatggcaat gcaggcgact cttttacctg gcacaacggc 1260  
 15 aaacagttta ccacctgga cagggaccat gatgtctaca caggaaactg tgcccactat 1320  
 cagaagggag gatggtggtta taacgcctgt gctcactcca acctcaatgg ggtctggtac 1380  
 cgtgggggcc attaccggag cagataccag gacggggtct actgggctga gttccgagga 1440  
 ggctcttact cactcaagaa ggtggtgatg atgattcggc ccaacccga tatcttccac 1500  
 taagctctcc ctgcctgg 1518

20 <210> 5  
 <211> 25  
 <212> DNA  
 <213> Artificial Sequence

25 <220>  
 <223> Description of Artificial Sequence: Antisense  
 nucleotide sequence against zebrafish  
 angiopoietin-like 1 precursor

30 <400> 5  
 acatccagac ctgaggacac atagc 25

35 <210> 6  
 <211> 25  
 <212> DNA  
 <213> Artificial Sequence

40 <220>  
 <223> Description of Artificial Sequence: Antisense  
 nucleotide sequence against zebrafish angiopoietin

40 <400> 6  
 atggctttct gggatgagac tcagg 25

45 <210> 7  
 <211> 491  
 <212> PRT  
 <213> Homo sapiens

50 <400> 7  
 Met Lys Thr Phe Thr Trp Thr Leu Gly Val Leu Phe Phe Leu Leu Val  
 1 5 10 15

Asp Thr Gly His Cys Arg Gly Gly Gln Phe Lys Ile Lys Lys Ile Asn  
 20 25 30

55 Gln Arg Arg Tyr Pro Arg Ala Thr Asp Gly Lys Glu Glu Ala Lys Lys

## EP 1 308 511 A1

	35	40	45
5	Cys Ala Tyr Thr Phe Leu Val Pro Glu Gln Arg Ile Thr Gly Pro Ile 50 55 60		
	Cys Val Asn Thr Lys Gly Gln Asp Ala Ser Thr Ile Lys Asp Met Ile 65 70 75 80		
10	Thr Arg Met Asp Leu Glu Asn Leu Lys Asp Val Leu Ser Arg Gln Lys 85 90 95		
	Arg Glu Ile Asp Val Leu Gln Leu Val Val Asp Val Asp Gly Asn Ile 100 105 110		
15	Val Asn Glu Val Lys Leu Leu Arg Lys Glu Ser Arg Asn Met Asn Ser 115 120 125		
	Arg Val Thr Gln Leu Tyr Met Gln Leu Leu His Glu Ile Ile Arg Lys 130 135 140		
20	Arg Asp Asn Ser Leu Glu Leu Ser Gln Leu Glu Asn Lys Ile Leu Asn 145 150 155 160		
	Val Thr Thr Glu Met Leu Lys Met Ala Thr Arg Tyr Arg Glu Leu Glu 165 170 175		
25	Val Lys Tyr Ala Ser Leu Thr Asp Leu Val Asn Asn Gln Ser Val Met 180 185 190		
	Ile Thr Leu Leu Glu Glu Gln Cys Leu Arg Ile Phe Ser Arg Gln Asp 195 200 205		
30	Thr His Val Ser Pro Pro Leu Val Gln Val Val Pro Gln His Ile Pro 210 215 220		
	Asn Ser Gln Gln Tyr Thr Pro Gly Leu Leu Gly Gly Asn Glu Ile Gln 225 230 235 240		
35	Arg Asp Pro Gly Tyr Pro Arg Asp Leu Met Pro Pro Pro Asp Leu Ala 245 250 255		
	Thr Ser Pro Thr Lys Ser Pro Phe Lys Ile Pro Pro Val Thr Phe Ile 260 265 270		
40	Asn Glu Gly Pro Phe Lys Asp Cys Gln Gln Ala Lys Glu Ala Gly His 275 280 285		
	Ser Val Ser Gly Ile Tyr Met Ile Lys Pro Glu Asn Ser Asn Gly Pro 290 295 300		
45	Met Gln Leu Trp Cys Glu Asn Ser Leu Asp Pro Gly Gly Trp Thr Val 305 310 315 320		
	Ile Gln Lys Arg Thr Asp Gly Ser Val Asn Phe Phe Arg Asn Trp Glu 325 330 335		
50	Asn Tyr Lys Lys Gly Phe Gly Asn Ile Asp Gly Glu Tyr Trp Leu Gly 340 345 350		
	Leu Glu Asn Ile Tyr Met Leu Ser Asn Gln Asp Asn Tyr Lys Leu Leu 355 360 365		



EP 1 308 511 A1

5 Ile Glu Leu Glu Asp Trp Ser Asp Lys Lys Val Tyr Ala Glu Tyr Ser  
 370 375 380  
 Ser Phe Arg Leu Glu Pro Glu Ser Glu Phe Tyr Arg Leu Arg Leu Gly  
 385 390 395 400  
 10 Thr Tyr Gln Gly Asn Ala Gly Asp Ser Met Met Trp His Asn Gly Lys  
 405 410 415  
 Gln Phe Thr Thr Leu Asp Arg Asp Lys Asp Met Tyr Ala Gly Asn Cys  
 420 425 430  
 15 Ala His Phe His Lys Gly Gly Trp Trp Tyr Asn Ala Cys Ala His Ser  
 435 440 445  
 Asn Leu Asn Gly Val Trp Tyr Arg Gly Gly His Tyr Arg Ser Lys His  
 450 455 460  
 20 Gln Asp Gly Ile Phe Trp Ala Glu Tyr Arg Gly Gly Ser Tyr Ser Leu  
 465 470 475 480  
 Arg Ala Val Gln Met Met Ile Lys Pro Ile Asp  
 485 490  
 25 <210> 8  
 <211> 493  
 <212> PRT  
 <213> Homo sapiens  
 30 <400> 8  
 Met Arg Pro Leu Cys Val Thr Cys Trp Trp Leu Gly Leu Leu Ala Ala  
 1 5 10 15  
 Met Gly Ala Val Ala Gly Gln Glu Asp Gly Phe Glu Gly Thr Glu Glu  
 20 25 30  
 35 Gly Ser Pro Arg Glu Phe Ile Tyr Leu Asn Arg Tyr Lys Arg Ala Gly  
 35 40 45  
 Glu Ser Gln Asp Lys Cys Thr Tyr Thr Phe Ile Val Pro Gln Gln Arg  
 50 55 60  
 40 Val Thr Gly Ala Ile Cys Val Asn Ser Lys Glu Pro Glu Val Leu Leu  
 65 70 75 80  
 Glu Asn Arg Val His Lys Gln Glu Leu Glu Leu Leu Asn Asn Glu Leu  
 85 90 95  
 45 Leu Lys Gln Lys Arg Gln Ile Glu Thr Leu Gln Gln Leu Val Glu Val  
 100 105 110  
 Asp Gly Gly Ile Val Ser Glu Val Lys Leu Leu Arg Lys Glu Ser Arg  
 115 120 125  
 50 Asn Met Asn Ser Arg Val Thr Gln Leu Tyr Met Gln Leu Leu His Glu  
 130 135 140  
 55 Ile Ile Arg Lys Arg Asp Asn Ala Leu Glu Leu Ser Gln Leu Glu Asn  
 145 150 155 160

EP 1 308 511 A1

5	Arg	Ile	Leu	Asn	Gln	Thr	Ala	Asp	Met	Leu	Gln	Leu	Ala	Ser	Lys	Tyr	
				165						170					175		
	Lys	Asp	Leu	Glu	His	Lys	Tyr	Gln	His	Leu	Ala	Thr	Leu	Ala	His	Asn	
			180					185						190			
10	Gln	Ser	Glu	Ile	Ile	Ala	Gln	Leu	Glu	Glu	His	Cys	Gln	Arg	Val	Pro	
			195					200					205				
	Ser	Ala	Arg	Pro	Val	Pro	Gln	Pro	Pro	Pro	Ala	Ala	Pro	Pro	Arg	Val	
		210					215					220					
15	Tyr	Gln	Pro	Pro	Thr	Tyr	Asn	Arg	Ile	Ile	Asn	Gln	Ile	Ser	Thr	Asn	
	225					230					235					240	
	Glu	Ile	Gln	Ser	Asp	Gln	Asn	Leu	Lys	Val	Leu	Pro	Pro	Pro	Leu	Pro	
					245					250					255		
20	Thr	Met	Pro	Thr	Leu	Thr	Ser	Leu	Pro	Ser	Ser	Thr	Asp	Lys	Pro	Ser	
				260					265					270			
	Gly	Pro	Trp	Arg	Asp	Cys	Leu	Gln	Ala	Leu	Glu	Asp	Gly	His	Asp	Thr	
			275					280					285				
25	Ser	Ser	Ile	Tyr	Leu	Val	Lys	Pro	Glu	Asn	Thr	Asn	Arg	Leu	Met	Gln	
		290					295					300					
	Val	Trp	Cys	Asp	Gln	Arg	His	Asp	Pro	Gly	Gly	Trp	Thr	Val	Ile	Gln	
	305					310					315					320	
30	Arg	Arg	Leu	Asp	Gly	Ser	Val	Asn	Phe	Phe	Arg	Asn	Trp	Glu	Thr	Tyr	
					325					330					335		
	Lys	Gln	Gly	Phe	Gly	Asn	Ile	Asp	Gly	Glu	Tyr	Trp	Leu	Gly	Leu	Glu	
			340						345					350			
35	Asn	Ile	Tyr	Trp	Leu	Thr	Asn	Gln	Gly	Asn	Tyr	Lys	Leu	Leu	Val	Thr	
			355					360					365				
	Met	Glu	Asp	Trp	Ser	Gly	Arg	Lys	Val	Phe	Ala	Glu	Tyr	Ala	Ser	Phe	
		370					375					380					
40	Arg	Leu	Glu	Pro	Glu	Ser	Glu	Tyr	Tyr	Lys	Leu	Arg	Leu	Gly	Arg	Tyr	
	385					390					395					400	
	His	Gly	Asn	Ala	Gly	Asp	Ser	Phe	Thr	Trp	His	Asn	Gly	Lys	Gln	Phe	
				405						410					415		
45	Thr	Thr	Leu	Asp	Arg	Asp	His	Asp	Val	Tyr	Thr	Gly	Asn	Cys	Ala	His	
			420						425					430			
	Tyr	Gln	Lys	Gly	Gly	Trp	Trp	Tyr	Asn	Ala	Cys	Ala	His	Ser	Asn	Leu	
			435					440					445				
50	Asn	Gly	Val	Trp	Tyr	Arg	Gly	Gly	His	Tyr	Arg	Ser	Arg	Tyr	Gln	Asp	
		450					455					460					
	Gly	Val	Tyr	Trp	Ala	Glu	Phe	Arg	Gly	Gly	Ser	Tyr	Ser	Leu	Lys	Lys	
	465					470					475					480	

# EP 1 308 511 A1

Val Val Met Met Ile Arg Pro Asn Pro Asn Thr Phe His  
485 490

5

<210> 9  
<211> 8  
<212> PRT  
<213> Artificial Sequence

10

<220>  
<221> VARIANT  
<222> (1)  
<223> Xaa is Asp or Asn

15

<220>  
<221> VARIANT  
<222> (2)  
<223> Xaa is Lys or His

20

<220>  
<221> VARIANT  
<222> (4)  
<223> Xaa is Val or Met

25

<220>  
<221> VARIANT  
<222> (6)  
<223> Xaa is Ala or Thr

30

<220>  
<221> VARIANT  
<222> (7)  
<223> Xaa is Gly or Arg

<220>  
<223> Description of Artificial Sequence: Cabinding motif

35

<400> 9  
Xaa Xaa Asp Xaa Tyr Xaa Xaa Asn  
1 5

40

<210> 10  
<211> 12  
<212> PRT  
<213> Artificial Sequence

45

<220>  
<221> VARIANT  
<222> (1)  
<223> Xaa is Trp or Cys

50

<220>  
<221> VARIANT  
<222> (2)  
<223> Xaa is Trp or Glu

55

<220>  
<221> VARIANT  
<222> (3)



<223> Xaa is Tyr or Leu

<220>

<223> Description of Artificial Sequence: Fibrinogen  
beta and gamma chains C-terminal domain

<400> 10

Xaa Xaa Xaa Asn Ala Cys Ala His Ser Asn Leu Asn  
1 5 10

<210> 11

<211> 21

<212> PRT

<213> Homo sapiens

<400> 11

Met Lys Thr Phe Thr Trp Thr Leu Gly Val Leu Phe Phe Leu Leu Val  
1 5 10 15

Asp Thr Gly His Cys  
20

## References

[0171] Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D.J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search Programs. *Nucleic Acids Res.* 25, 3389-3402.

[0172] Amatruda, J.F., and Zon, L.I. (1999). Dissecting hematopoiesis and disease using the zebrafish. *Dev. Biol.* 216, 1-15.

[0173] Bemis, L.T., Geske, F.J., and Strange, R. (1995). Use of the yeast two-hybrid system for identifying the cascade of protein interactions resulting in apoptotic cell death. *Methods Cell Biol.* 46, 139-51

[0174] Benton, W.D. and Davis, R.W. (1977). Screening  $\lambda$ gt recombinant clones by hybridization to single plaques in situ. *Science* 196, 180-182

[0175] Bindseil, K.U., Jakupovic, J., Wolf, D., Lavayre, J., Leboul, J., and van der Pyl, D. (2001). Pure compound libraries; a new perspective for natural product based drug discovery. *Drug Discov. Today* 6, 840-847.

[0176] Bradley, A., Evans, M., Kaufman, M.H., Robertson, E. (1984). Formation of germ-line chimaeras from embryo-derived teratocarcinoma cell lines. *Nature* 309, 255-256.

[0177] Breier, G., Clauss, M., and Risau, W. (1995). Coordinate expression of vascular endothelial growth factor receptor-1 (flt-1) and its ligand suggests a paracrine regulation of murine vascular development. *Dev. Dyn.* 204, 228-239.

[0178] Brinster, R.L., Chen, H.Y., Trumbauer, M.E., Yagle, M.K., Palmiter, R.D. (1985). Factors affecting the efficiency of introducing foreign DNA into mice by microinjecting eggs. *Proc. Natl. Acad. Sci. USA* 82, 4438-4442.

[0179] Brown, L.A., Rodaway, A.R., Schilling, T.F., Jowett, T., Ingham, P.W., Patient, R.K., and Sharrocks, A.D. (2000). Insights into early vasculogenesis revealed by expression of the ETS-domain transcription factor Fli-1 in wild type and mutant zebrafish embryos. *Mech. Dev.* 90, 237-252.

[0180] Claesson-Welsh, L. (ed.) (1999). *Current Topics in Microbiology and Immunology*. (Berlin: Springer Verlag, GmbH & Co), 237, 1-187.

[0181] Conklin, D., Gilbertson, D., Taft, D.W., Maurer, M.F., Whitmore, T.E., Smith, D.L., Walker, K.M., Chen, L.H., Wattler, S., Nehls, M., and Lewis, K.B. (1999). Identification of a mammalian angiopoietin-related protein expressed specifically in liver. *Genomics* 62, 477-482.

[0182] Cull M. G., Miller, J.F., and Schatz, P.J. (1992). Screening for receptor ligands using large libraries of peptides linked to the C terminus of the *lac* repressor. *Proc. Natl. Acad. Sci. USA* 89, 1865-1869.

[0183] Cwirla, S.E., Peters, E. A., Barrett, R.W., and Dower, W.J. (1990). Peptides on phage: a vast library of peptides for identifying ligands. *Proc. Natl. Acad. Sci. USA* 87, 6378-6382.

[0184] Davis, S., Aldrich, T.H., Jones, P.F., Acheson, A., Compton, D.L., Jain, V., Ryan, T.E., Bruno, J., Radziewski, C., Maisonpierre, P.C., and Yancopoulos, G.D. (1996). Isolation of angiopoietin-1, a ligand for the TIE2 receptor, by

secretion-trap expression cloning. *Cell*. 87, 1161-1169.

[0185] Detrich III, H.W., Kieran, M.W., Chan, F.Y., Barone, L.M., Yee, K., Rundstadler, J.A., Pratt, S., Ransom, D., and Zon, L.I. (1995). Intraembryonic hematopoietic cell migration during vertebrate development. *Proc. Natl. Acad. Sci. USA* 92, 10713-10717.

5 [0186] Devlin, J.J., Panganiban, L.C., and Devlin, P.E. (1990) Random peptide libraries: a source of specific protein binding molecules. *Science* 249, 404-406.

[0187] de Vries, C., Escobedo, J.A., Ueno, H., Houck, K., Ferrara, N., and Williams, L.T. (1992). The fins-like tyrosine kinase, a receptor for vascular endothelial growth factor. *Science* 255, 989-991.

10 [0188] DeWitt, S. H., Kiely, J.S., Stankovic, C.J., Schroeder, M.C., Reynolds Cody, D.M., and Pavia, M.R. (1993). "Diversomers": An approach to nonpeptide, nonoligomeric chemical diversity. *Proc. Natl. Acad. Sci. USA* 90, 6909-6913.

[0189] Doetschman, T.C., Eistetter, H., Katz, M., Schmidt, W., and Kemler, R. (1985). The in vitro development of blastocyst-derived embryonic stem cell lines: formation of visceral yolk sac, blood islands and myocardium. *J. Embryol. Exp. Morphol.* 87, 27-45.

15 [0190] Ekker, S.C. (2000). Morphants: a new systematic vertebrate functional genomics approach. *Yeast* 17, 302-306.

[0191] Endo, T., Kitajima, Y., Nishikawa, A., Manase K., Shibuya, M., and Kudo, R. (2001). Cyclic changes in expression of mRNA of vascular endothelial growth factor, its receptors Flt-1 and KDR/Flk-1, and Ets-1 in human corpora lutea. *Fertil. Steril.* 76, 762-768.

20 [0192] Erb, E., Janda, K.D., and Brenner, S. (1994). Recursive deconvolution of combinatorial chemical libraries. *Proc. Natl. Acad. Sci. USA* 91, 11422-11426.

[0193] Evans, M.J., and Kaufman, M.H. (1981). Establishment in culture of pluripotential cells from mouse embryos. *Nature* 292, 154-156.

25 [0194] Felici, F., Castagnoli, L., Musacchio, A., Jappelli, R., and Cesareni, G. (1991). Selection of antibody ligands from a large library of oligopeptides expressed on a multivalent expression vector. *J. Mol. Biol.* 222, 301-310.

[0195] Fields, S., and Sternglanz, R. (1994). The two-hybrid system: an assay for protein-protein interactions. *Trends Genet.* 10, 286-292.

[0196] Fodor, S.P., Rava, R.P., Huang, X.C., Pease, A.C., Holmes, C.P., and Adams, C.L. (1993). Multiplexed biochemical assays with biological chips. *Nature* 364, 555-556.

30 [0197] Fong, G-H., Rossant, J., Gertsenstein, M., and Breitman, M.L. (1995). Role of the flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium. *Nature* 376, 66-70.

[0198] Froehler, B., Ng, P., and Matteucci, M. (1988). Phosphoramidate analogues of DNA: synthesis and thermal stability of heteroduplexes. *Nucleic Acids Res.* 16, 4831-4839.

35 [0199] Galland, F., Karamysheva, A., Pebusque, M.J., Borg, J.P., Rottapel, R., Dubreuil P., Rosnet, O., and Bimbaum, D. (1993). The FLT4 gene encodes a transmembrane tyrosine kinase related to the vascular endothelial growth factor receptor. *Oncogene* 8, 1233-1240.

[0200] Gallop, M.A., Barrett, R.W., Dower, W.J., Fodor, S.P., and Gordon, E.M. (1994). Applications of combinatorial technologies to drug discovery. 1. Background and peptide combinatorial libraries. *J. Med. Chem.* 37: 1233-1251.

40 [0201] Gordon, E.M., Barrett, R.W., Dower, W.J., Fodor, S.P., and Gallop, M.A. (1994). Applications of combinatorial technologies to drug discovery. II. Combinatorial organic synthesis, library screening strategies and future directions. *J. Med. Chem.* 37, 1385-1401.

[0202] Gossler, A., Doetschman, T., Kom, R., Serfling, E., Kemler, R (1986). Transgenesis by means of blastocyst-derived embryonic stem cell lines. *Proc. Natl. Acad. Sci. USA* 83, 9065-9069.

45 [0203] Grabley, S., Thiericke, R., and Sattler, I. (2000). Tools for drug discovery: natural product-based libraries. *Ernst Schering Res. Found. Workshop.* 32, 217-252.

[0204] Hamaguchi, I., Huang, X-L., Takakura, N., Tada, J-I., Yamaguchi, Y., Hiroaki, K., and Suda, T. (1999). In vitro hematopoietic and endothelial cell development from cells expressing TEK receptor in murine aorta-gonad-mesonephros region. *Blood* 93, 1549-1556.

50 [0205] Hattori, K., Dias, S., Heissig, B., Hackett, N.R., Lynden, D., Tateno, M., Hicklin, D.J., Zhu, Z., Witte, L., Crystal, R.G., Moore, M.A., and Rafii, S. (2001). Vascular endothelial growth factor and angiopoietin-1 stimulate postnatal hematopoiesis by recruitment of vasculogenic and hematopoietic stem cells. *J. Exp. Med.* 193, 1005-1014.

[0206] Hauptmann, G. (1999). Two-color detection of mRNA transcript localizations in fish and fly embryos using alkaline phosphatase and  $\beta$ -galactosidase conjugated antibodies. *Dev. Genes. Evol.* 209, 317-321.

55 [0207] Houghten, R.A., Wilson, D.B., and Pinilla, C. (2000). Drug discovery and vaccine development using mixture-based synthetic combinatorial libraries. *Drug Discov. Today* 5, 276-285.

[0208] Houghten, R.A., Appel, J.R., Blondelle, S.E., Cuervo, J.H., Dooley, C.T, and Piunilla, C. (1992). The use of synthetic peptide combinatorial libraries for the identification of bioactive peptides. *Biotechniques.* 13, 412-421.

[0209] Huang, H., Vogel, S.S., Liu, N., Melton, D.A., and Lin, S. (2001). Analysis of pancreatic development in living

transgenic zebrafish embryos. *Mol. Cell. Endocrinol.* **177**, 117-124.

[0210] Huang, X.L., Takakura, N., and Suda, T. (1999). In vitro effects-of angiopoietins and VEGF on hematopoietic and endothelial cells. *Biochem. Biophys. Res. Commun.* **264**, 133-138.

[0211] Hyrup, B., and Nielsen, P.E. (1996). Peptide nucleic acids (PNA): synthesis, properties and potential applications. *Bioorg. Med. Chem.* **4**, 5-23.

[0212] Jaenisch, R. (1988). Transgenic animals. *Science*. **240**, 1468-1474.

[0213] Jaenisch, R. (1976). Germ line integration and Mendelian transmission of the exogenous Moloney leukemia virus. *Proc Natl Acad Sci. USA* **73**, 1260-1264.

[0214] Jahner, D., Stuhlmann, H., Stewart, C.L., Harbers, K., Lohler, J., Simon, I., and Jaenisch, R. (1982). De novo methylation and expression of retroviral genomes during mouse embryogenesis. *Nature* **298**, 623-628.

[0215] Jahner, D., Haase, K., Mulligan, R., and Jaenisch R. (1985). Insertion of the bacterial gpt gene into the germ line of mice by retroviral infection. *Proc. Natl. Acad. Sci. USA* **82**, 6927-31.

[0216] Jowett, T. (2001). Double in situ hybridisation techniques. *Methods* **23**, 345-358.

[0217] Kaipainen, A., Korhonen, J., Mustonen, T., van Hinsbergh, V.W., Fang, G.H., Dumont, D., Breitman, M., and Alitalo, K. (1995). Expression of the fins-like tyrosine kinase 4 gene becomes restricted to lymphatic endothelium during development. *Proc. Natl. Acad. Sci. USA* **92**, 3566-3570.

[0218] Khoury, G., and Gruss, P. (1983). Enhancer Elements. *Cell* **33**, 313-314.

[0219] Kim, I., Kwak, H.J., Ahn, J.E., So, J-N., Liu, M., Koh, K.N., and Koh, G.Y. (1999a). Molecular cloning and characterization of a novel angiopoietin family protein, angiopoietin-3. *FEBS Lett.* **443**, 353-356.

[0220] Kim, I., Moon, S-O., Koh, K.N., Kim, H., Uhm, C-S., Kwak, H.J., Kim, N-G., Koh, G.Y. (1999b). Molecular cloning, expression and characterization of angiopoietin-related protein. *J. Biol. Chem.* **274**, 26523-26528.

[0221] Kim, I., Kim, H.G., So, J-N., Kim, J.H., Kwak, H.J., and Koh, G.Y. (2000). Angiopoietin-1 regulates endothelial cell survival through the phosphatidylinositol 3'-kinase/Akt signal transduction pathway. *Circ. Res.* **86**, 24-29.

[0222] Kontos, C.D., Stauffer, T.P., Yang, W.P., York, J.D., Huang, L., Blannar, M.A., Meyer, T., and Peters, K.G. (1998). Tyrosine 1101 of Tie2 in the major site of association of p85 and is required for activation of phosphatidylinositol 3-kinase and Akt. *Mol. Cell Biol.* **18**, 4131-4140.

[0223] Koster, R.W., and Fraser, S.E. (2001). Tracing transgene expression in living zebrafish embryos. *Dev. Biol.* **233**, 329-346

[0224] Kwak, H.J., So, J-N., Lee, S.J., Kim, I., and Koh, G.Y. (1999). Angiopoietin-1 is an apoptosis survival factor for endothelial cells. *FEBS Lett.* **448**, 249-253.

[0225] Lakso, M., Sauer, B., Mosinger, B. Jr., Lee, E.J., Manning, R.W., Zu, S.H., Mulder, K.L., and Westphal, H. (1992). Targeted oncogene activation by site-specific recombination in transgenic mice. *Proc. Natl. Acad. Sci. USA* **89**, 6232-6236.

[0226] Lam, K.S., Salmon, S.E., Hersh, E.M., Hruby, V.J., Kazmierski, W.M., and Knapp, R.J. (1991). A new type of synthetic peptide library for identifying ligand-binding activity. *Nature* **354**, 82-84.

[0227] Lemaitre, M., Bayard, B., and Lebleu, B. (1987). Specific antiviral activity of a poly (L-lysine)-conjugated oligodeoxyribonucleotide sequence complementary to vesicular stomatitis virus N protein mRNA initiation site. *Proc. Natl. Acad. Sci. USA* **84**, 648-652.

[0228] Letsinger, R.L., Zhang, G.R., Sun, D.K., Ikeuchi, T., and Sarin, P.S. (1989). Cholesteryl-conjugated oligonucleotides: synthesis, properties, and activity as inhibitors of replication of human immunodeficiency virus in cell culture. *Proc. Natl. Acad. Sci. USA* **86**, 6553-6556.

[0229] Liang, D., Chang, J.R., Chin, A.J., Smith, A., Kelly, C., Weinberg, E.S., Ge, R. (2001). The role of vascular endothelial growth factor (VEGF) in vasculogenesis, angiogenesis, and hematopoiesis in zebrafish development. *Mech. Dev.* **108**, 29-43.

[0230] Long, Q., Meng, A., Wang, H., Jessen, J.R., Farrell, M.J., and Lin, S. (1997). GATA-1 expression pattern can be recapitulated in living transgenic zebrafish using GFP reporter gene. *Development*. **124**, 4105-4111.

[0231] Lyons, M.S., Bell, b., Stainier, D., and Peters, K.G. (1998). Isolation of the zebrafish homologues for the tie-1 and tie-2 endothelium-specific receptor tyrosine kinases. *Dev. Dyn.* **212**, 133-140.

[0232] Meng, A., Jessen, J.R. and Lin, S. (1999). Transgenesis. *Methods Cell Biol.* **60**, 133-148.

[0233] Maisonpierre, P.C., Suri, C., Jones, P.F., Bartunkova, S., Wiegand, S.J., Radziejewski, C., Compton, D., McClain, J., Aldrich, T.H., Papadopoulos, N., Daly, T.J., Davis, S., Sato, T.N., and Yancopoulos, G.D. (1997). Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. *Science* **277**, 55-60.

[0234] Millauer, B., Witzmann-Voos, S., Schnurch, H., Martinez, R., Moller, N.P., Risau, W., and Ullrich, A. (1993). High affinity VEGF binding and developmental expression suggest Flk-1 as a major regulator of vasculogenesis and angiogenesis. *Cell* **72**, 835-46.

[0235] Miller. (1989). Oligonucleotides: Antisense Inhibitors of Gene Expression: a formacetal or analog thereof, as well as  $\alpha$ -DNA. pp. 82-85.

[0236] Minden, A., Lin, A., Smeal, T., Derijard, B., Cobb, M., Davis, R., and Karin, M. (1994). C-Jun N-terminal phos-



phorylation correlates with activation of the JNK subgroup but not the ERK subgroup of mitogen-activated protein kinases. *Mol. Cell. Biol.* **14**, 6683-6688.

[0237] Motoike, T., Loughna, S., Perens, E., Roman, B.L., Liao, W., Chau, T.C., Richardson, C.D., Kawate, T., Kuno, J., Weinstein, B.M., Stainier, D.Y., and Sato, T.N. (2000). Universal GFP reporter for the study of vascular development. *Genesis* **28**, 75-81

[0238] Mustonen, T., and Alitalo, K. (1995). Endothelial receptor tyrosine kinase involved in angiogenesis. *J. Cell Biol.* **129**, 895-898.

[0239] Nasevicius, A., and Ekker, S.C. (2000). Effective targeted gene 'knockdown' in zebrafish. *Nat. Genet.* **26**, 216-220.

[0240] Neufeld, G., Cohen, T., Gengrinovitch, S., and Poltoral, Z. (1999). Vascular endothelial growth factor (VEGF) and its receptors. *FASEB J.* **13**, 9-22.

[0241] Nielsen, P.E. (2000). Antisense peptide nucleic acids. *Curr. Opin. Mol. Ther.* **2**, 282-287.

[0242] O'Gorman, S., Fox, D.T., and Wahl, G.M. (1991). Recombinase-mediated gene activation and site-specific integration in mammalian cells. *Science* **251**, 1351-1355.

[0243] Orban, P.C., Chui, D., and Marth, J.D. (1992). Tissue- and site-specific DNA recombination in transgenic mice. *Proc. Natl. Acad. Sci. USA* **89**, 6861-6865.

[0244] Orum, H., and Wengel, J. (2001). Locked nucleic acids: a promising molecular family for genefunction analysis and antisense drug development. *Curr. Opin. Mol. Ther.* **3**, 239-243.

[0245] Palmer, A., Gavin, A.C., and Nebreda, A.R. (1998). A link between MAP kinase and p34<sup>cdc2</sup>/cyclin b during oocyte maturation: p90<sup>rsk</sup> phosphorylates and inactivates the p34<sup>cdc2</sup> inhibitory kinase Mytl. *EMBO J.* **17**, 5037-5047.

[0246] Pham, A.N., Roman, B.L., and Weinstein, B.M. (2001). Isolation and expression analysis of three zebrafish angiopoietin genes. *Dev. Dyn.* **221**, 470-474.

[0247] Procopio, W.N., Pelavin, P.I., Lee, W.M.F., and Yielding, N.M. (1999). Angiopoietin-1 and -2 coiled coil domains mediate distinct homo-oligomerization patterns, but fibrinogen-like domains mediate ligand activity. *J. Biol. Chem.* **274**, 30196-30201.

[0248] Rader, C. (2001). Antibody libraries in drug and target discovery. *Drug Discov. Today* **6**, 36-43.

[0249] Rayner, B., Malvy, C., Paolem, J., Lebleu, B., Paoletti, C., and Imbach, J. (1989).  $\alpha$ -oligodeoxynucleotide analogues. In *Oligonucleotides: Antisense Inhibitors of Gene Expression*. J. Cohen, ed. (Boca Raton, FL: CRC Press, Inc.), pp.119-136.

[0250] Risau, W. (1991). Vasculogenesis, angiogenesis and endothelial cell differentiation during embryonic development. In *The development of the vascular system*, R.N. Feinberg, G.K. Shere, and R. Auerbach, eds. (Basel: Karger), pp. 58-68.

[0251] Risau, W. (1997). Mechanisms of angiogenesis. *Nature* **386**, 671-674.

[0252] Robertson, E., Bradley, A., Kuehn, M., and Evans, M. (1986). Germ-line transmission of genes introduced into cultured pluripotential cells by retroviral vector. *Nature* **323**, 445-448.

[0253] Rodriguez, R., and Chamberlin, M. (eds.) (1982). *Promoters: Structure and Function*. (New York: Praeger).

[0254] Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B., and Erlich, H.A. (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **239**, 487-491.

[0255] Saragovi, H.U., Greene, M.I., Chrusciel, R.A., and Kahn, M. (1992). Loops and secondary structure mimetics: development and applications in basic science and rational drug design. *Biotechnology* **10**, 773-778.

[0256] Sarin, P.S., Agrawal, S., Civeira, M.P., Goodchild, J., Ikeuchi, T., and Zamecnik, P.C. (1988). Inhibition of acquired immunodeficiency syndrome virus by oligodeoxynucleoside methylphosphonates. *Proc. Natl. Acad. Sci. USA* **85**, 7448-7451.

[0257] Scott, J.K., and Smith, G.P. (1990). Searching for peptide ligands with an epitope library. *Science* **249**, 386-390.

[0258] Schaefer, B. (1995). Revolution in rapid amplification of cDNA ends: new strategies for polymerase chain reaction cloning of full-length cDNA ends. *Anal. Biochem.* **227**, 255-273

[0259] Shalaby, F., Rossant, J., Yamaguchi, T.P., Gertenstein, M., Wu, X-F., Breitman, M.L., and Schuh, A.C. (1995). Failure of blood island formation and vasculogenesis in flk-1-deficient mice. *Nature* **376**, 62-66.

[0260] Shibahara, S., Mukai, S., Morisawa, H., Nakashima, H., Kobayashi, S., and Yamamoto, N. (1989). Inhibition of human immunodeficiency virus (HTV-1) replication by synthetic oligo-RNA derivatives. *Nucleic Acids Res.* **17**, 239-252.

[0261] Shibuya, M., Yamaguchi, S., Yamane, A., Ikeda, T., Tojo, A., Matsushime, H., and Sato, M. (1990). Nucleotide sequence and expression of a novel human receptor-type tyrosine kinase gene (fit) closely related to the fins family. *Oncogene* **5**, 519-524.

[0262] Stein, C., and Cohen, J. (1989). Phosphorothioate oligonucleotide analogs. In *Oligonucleotides: Antisense Inhibitors of Gene Expression*. J. Cohen, ed. (Boca Raton, FL: CRC Press, Inc.), pp.97-117.

[0263] Stein, C.A., Subasinghe, C., Shinozuka, K., and Cohen, J.S. (1988). Physicochemical properties of phospho-

rothioate oligodeoxynucleotides. Nucleic Acids Res. 16, 3209-3221.

[0264] Summerton, J. (1999). Morpholino antisense oligomers: the case for an RNase H-independent structural type. Biochim. Biophys. Acta. 1489, 141-158.

[0265] Summerton, J., and Weller, D. (1997). Morpholino antisense oligomers: design, preparation, and properties. Antisense Nucleic Acid Drug Dev. 7, 187-195.

[0266] Takakura, N., Huang, X.L., Naruse, T., Hamaguchi, I., Dumont, D.J., Yancopoulos, G.D., and Suda, T. (1998). Critical role of the TIE2 endothelial cell receptor in the development of definitive hematopoiesis. Immunity 9, 677-686.

[0267] Terman, B.I., Dougher-vermazen, M., Carrion, M.E., Dimitrov, D., Armellino, D.C., Gospodarowicz, D., and Bohlen, P. (1992). Identification of the KDR tyrosine kinase as a receptor for vascular endothelial cell growth factor. Biochem. Biophys. Res. Commun. 187, 1579-1586.

[0268] Thompson, M.A., Ransom, D.G., Pratt, S.J., MacLennan, H., Kieran, M.W., Detrich, H.W., Vail, B., Huber, T. L., Paw, B., Brownlie, A.J., Oates, A.C., Fritz, A., Gates, M.A., Amores, A., Bahary, N., Talbot, W.S., Her, H., Beier, D. R, Postlethwait, J.H., and Zon, L.I. (1998). The *cloche* and *spadetail* genes differentially affect hematopoiesis and vasculogenesis. Dev. Biol. 197, 248-269.

[0269] Topcu, Z. and Borden, K.L. (2000). The yeast two-hybrid system and its pharmaceutical significance. Pharm Res. 17, 1049-55.

[0270] Valenzuela, D.m., Griffiths, J.A., Rojas, J., Aldrich, T.H., Jones, P.F., Zhou, H., McClain, J., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., Huang, T., Papadopoulos, N., Maisonpierre, P.C., Davis, S., and Yancopoulos, G.D. (1999). Angiopoietins 3 and 4: diverging gene counterparts in mice and humans. Proc. Natl. Acad. Sci. USA 96, 1904-1090.

[0271] van der Krol, A.R., Mol, J.N., and Stuitje, A.R. (1988). Modulation of eukaryotic gene expression by complementary RNA or DNA sequences. Biotechniques 6, 958-976.

[0272] van der Putten, H., Botteri, F.M., Miller, A.D., Rosenfeld, M.G., Fan, H., Evans, R.M., and Verma, I.M. (1985). Efficient insertion of genes into the mouse germ line via retroviral vectors. Proc. Natl. Acad. Sci. USA 82, 6148-6152.

[0273] Wagner, R. W. (1994). Gene inhibition using antisense oligodeoxynucleotides. Nature 372, 333-335

[0274] Wahlestedt, C., Salmi, P., Good, L., Kela, J., Johnsson, T., Hokfelt, T., Broberger, C., Porreca, F., Lai, J., Ren, K., Ossipoy, M., Koshkin, A., Jakobsen, N., Skouv, J., Oerum, H., Jacobsen, M.H., and Wengel, J. (2000). Potent and nontoxic antisense oligonucleotides containing locked nucleic acids. Proc. Natl. Acad. Sci. USA 97, 5633-5638.

[0275] Westerfield, M. (2000). The Zebrafish Book, Institute of Neuroscience, University of Oregon.

[0276] Xu, W-F., Chung, D.W., and Davie, W.E. (1996). The assmebly of human fibrinogen. J. Biol. Chem. 271, 27948-27953.

[0277] Zhang, B., Kraemer, B., SenGupta, D., Fields, S. and Wickens, M. (1999). Yeast three-hybrid system o detect and analyse interactions between RNA and protein. Methods Enzymol. 306, 93-113.

[0278] Zon, G. (1988). Oligonucleotide analogues as potential chemotherapeutic agents. Pharm Res. 5, 539-549.

ADDITIONAL REFERENCES

[0279] The Polymerase Chain Reaction. Current Protocols In Molecular Biology, John Wiley & Sons, N.Y. (1989), Vol-3, 15.0.3-15.8.7.

[0280] Preparation of Poly (A)<sup>+</sup> RNA. Current Protocols In Molecular Biology, John Wiley & Sons, N.Y. (1989), Vol-1, 4.5.1-4.5.3.

US Patent Documents:		
<u>4,683,195</u>	Mullis et al.	Jul., 1987
<u>4,833,080</u>	Brent et al.	May, 1989
<u>5,185,444</u>	Summerton et al.	Feb., 1993
<u>5,217,866</u>	Summerton et al.	Jun., 1993
<u>5,223,409</u>	Lander et al.	Jun., 1993
<u>5,403,484</u>	Lander et al.	April, 1995
<u>5,434,131</u>	Lindsley et al.	Jul., 1995
<u>5,571,698</u>	Lander et al.	Nov., 1996

Foreign Patent Documents:			
<u>89/10134</u>	Pardridge et al.	Nov., 1989	WO

(continued)

Foreign Patent Documents:			
88/09810	Tullis, R.	Feb., 1990	WO
92/15694	Wahl et al.	Sept., 1992	WO

**Other References Cited:**

- [0281] Kim et al., J Biol Chem (1999) Accession No. AF125176  
 [0282] Kim et al., J Biol Chem (1999) Accession No. NM\_012098  
 [0283] NCBI Annotation Project (2001) Accession No. XM\_001720  
 [0284] Sugano et al. unpublished (1999) Accession No. AW174181

**Claims**

1. A pharmaceutical composition comprising angiopoietin-like 1 (ANGPTL1) or angiopoietin-like 2 (ANGPTL2) protein, a fragment or a derivative thereof.
2. The composition of claim 1, wherein the composition comprises a polypeptide, which is at least 55% identical with the sequence shown in SEQ ID NO: 7 or SEQ ID NO: 8, a fragment or derivative thereof.
3. A pharmaceutical composition comprising a polynucleotide sequence encoding ANGPTL1 or ANGPTL2, a fragment, or a derivative thereof.
4. The composition of 3, wherein the polynucleotide sequence is at least 70% identical with a polynucleotide sequence which encodes the sequence shown in SEQ ID NO: 7 or SEQ ID NO: 8, a fragment or a derivative thereof.
5. The composition of 3, wherein the polynucleotide sequence is at least 70% identical with the polynucleotide sequence shown in SEQ ID NO: 2 or SEQ ID NO: 3.
6. The composition of any of claims 1-5, further comprising a suitable carrier.
7. The composition of claims 3-5, wherein the polynucleotide sequence is operably linked to an expression control sequence.
8. The composition of claim 7, wherein the polynucleotide sequence operably linked to an expression control sequence is in a host cell.
9. The composition of 8, wherein the host cell is a yeast, insect, teleost, amphibian or mammalian cell.
10. The composition of any of claims 3-9, wherein the polynucleotide sequence is in a delivery complex.
11. The composition of 10, wherein the delivery complex is a sterol, lipid or virus.
12. A method of screening for a therapeutic agent for the treatment of blood-related disorders or a defect in vasculature comprising:
  - a. contacting ANGPTL1 polypeptide, ANGPTL2 polypeptide, fragment or derivative thereof with a test compound or molecule or a library of test molecules or compounds, under conditions to allow specific binding and/interaction; and
  - b. detecting the level of specific binding and/interaction,
 wherein an alteration in the level of interaction relative to a control indicates a potential therapeutic activity.
13. A method of screening for a therapeutic agent for the treatment of blood-related disorders or a defect in vasculature comprising:



- a. contacting a reporter construct under the control of ANGPTL1 or ANGPTL2 promoter with a test molecule or compound or a library of test molecules or compounds, under conditions to allow specific binding and/ interaction; and
- b. detecting the level of expression of the reporter construct,

wherein an alteration in the level of expression to a control indicates a potential therapeutic activity.

**14.** The method of claims 12 and 13, wherein the library of test molecules or compounds is selected from the group consisting of DNA molecules, peptides, agonists, antagonists, monoclonal antibodies, immunoglobulins and pharmaceutical agents.

**15.** A method for treating blood-related disorders or defects in vasculature in a human patient comprising:

- a. providing a composition that comprises a therapeutic agent identified in the method of any one of claims 12-14; and
- b. administering a therapeutically effective amount of the composition from (a) to the patient.

**16.** The method of claim 15, wherein the test molecules or compounds are in a suitable carrier.

**17.** A method for treating blood-related disorders or defects in vasculature, comprising administering to a patient a therapeutically effective amount of a polypeptide comprising ANGPTL1 or ANGPTL2, or a fragment or a derivative thereof.

**18.** The method of claim 12 or 17, wherein the polypeptide is at least 55% identical with the sequence shown in SEQ ID NO: 7 or SEQ ID NO: 8, a fragment or derivative thereof.

**19.** A method for treating blood-related disorders or defects in vasculature, comprising administering to a patient a therapeutically effective amount of polynucleotide sequence encoding ANGPTL1 or ANGPTL2, or a fragment or a derivative thereof.

**20.** The method of claim 19, wherein the polynucleotide sequence is at least 70% identical with the polynucleotide sequence which encodes the sequence shown in SEQ ID NO: 7 or SEQ ID NO: 8, a fragment or a derivative thereof.

**21.** The method of any one of claims 12-20, wherein the blood-related disorder is leukemia or anemia.

**22.** A method for modulating proliferation and/or differentiation or cell death of target cells comprising administering a biologically active amount of an ANGPTL1 or ANGPTL2 polypeptide or polynucleotide to said target cells.

**23.** The method of claim 22, wherein said target cells are hematopoietic stem cells, erythroid or endothelial cells.

**24.** A method for determining whether a subject is at risk of developing or has a blood related disease or a defect in vasculature caused by angiopoietin-like proteins comprising determining the angiopoietin-like allele status of said subject; an altered angiopoietin-like allele status compared to a control indicating that said subject is at risk of developing or has a blood-related disease or a defect in vasculature caused by angiopoietin-like proteins.

**25.** A kit identifying a subject at risk of developing a blood-related disease or a defect in vasculature or having a blood-related disease or subject with a defect in vasculature caused by angiopoietin-like proteins, comprising means for measuring the level of an angiopoietin-like protein encoding polynucleotide sequence in a sample of cells of said subject and instructions to use the kit.

**26.** A transgenic non-human animal which does not produce functional ANGPTL1 and/or ANGPTL2 protein, or which produces sub-optimal levels of ANGPTL1 and/or ANGPTL2 protein, or which overproduces the ANGPTL1 and/or ANGPTL2 protein.

**27.** The animal of claim 26, wherein the genome of the animal comprises a mutation in the ANGPTL1 or ANGPTL2 genes.

**28.** The animal of claim 27, wherein said mutation is a homozygous disruption.

29. The animal of claim 27, wherein said mutation is a insertion, deletion, or a substitution mutation.
30. The animal of claim 27, wherein said mutation is generated by gene targeting, gene trap integration, or mutagenesis.
31. The animal of claim 27, wherein the mutation has occurred in an exon, intron, regulatory region or splicing site of the ANGPTL1 and/or ANGPTL2 genes.
32. The animal of any of the claims 26-31, wherein said animal exhibits a blood-related disease or a defect in vasculature.
33. The animal of any of the claims 26-32, wherein said animal is a mammal, amphibian, teleost or insect.
34. An antisense oligomer, wherein said oligomer is complementary to mRNA encoding ANGPTL1 or ANGPTL2, and wherein said antisense oligomer is from 6 to 50 nucleotides in length.
35. The oligomer of claim 34, wherein the antisense oligomer is complementary to the translation initiation site of ANGPTL1 or ANGPTL2.
36. The oligomer of claim 34, wherein said antisense oligomer is complementary to the polynucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4.
37. The oligomer of any of claims 34 to 36, wherein the antisense oligomer comprises the nucleotide sequence of SEQ ID: 5 or SEQ ID NO:6.
38. The oligomer of any of claims 34-37, wherein binding of said oligomer to said mRNA is effective in altering transcription or translation of said mRNA in a host cell expressing said mRNA.
39. The oligomer of claim 38, wherein the host cell is a bacterial, yeast, insect, or vertebrate cell.
40. The oligomer of any of claims 34-39, wherein the antisense oligomer is in a delivery complex.
41. The oligomer of claim 40, wherein the delivery complex is a sterol, lipid, virus.
42. The oligomer of claims 34-41, wherein said oligomer comprises:
  - a. at least one modified base moiety, preferably selected from the group consisting of 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl)uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5<sup>1</sup>-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), - 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine, and/or
  - b. at least one modified sugar moiety, preferably selected from the group consisting of arabinose, 2-fluoroarabinose, xylulose, and hexose, and/or
  - c. at least one modified phosphate backbone, preferably selected from the group consisting of phosphorothioate, phosphoramidothioate, phosphoramidate, phosphordiamidate, methylphosphonate, alkyl phosphotriester, formacetal or analog thereof, as well as  $\alpha$ -DNA, 2'-O-methyl RNA, and morpholine backbone.

**Figure 1**

fi40b08.y1 Sugano Kawakami zebrafish DRA Danio rerio cDNA clone 2640087 5' similar to  
TR:O95841 O95841 ANGIOPOIETIN Y1.

Length: 146 aa

```

5 atgtgtcctcaggtctggatgttctgctgggttcctctgtgtgctg
  M C P Q V W M F C W F L C V L
50 tgggtgtgtgcggatcagcagttcctccagccggcagcgcaggcag
  W C V R I S S S S S R Q R R Q
95 atggaggctccagaccgcagcggcagcagcagtgctcctacacc
  M E A P D P Q R Q Q Q C S Y T
140 ttcttaatcccggagcagaagatcacggggcctatctgtgccagc
  F L I P E Q K I T G P I C A S
185 cggggggccctcgctgccggaccccgagcgcgtgacgcggacggac
  R G P S L P D P E R V T R T D
230 atgtctgagctgcggcagaccctgagccggcagcggcgggagatg
  M S E L R Q T L S R Q R R E M
275 gagacgctgcggatgatggtggatgtggacggagacatggtgcag
  E T L R M M V D V D G D M V Q
320 gagatgaagctgctgcgtaaggagtcgcggaacatgaactcgcgg
  E M K L L R K E S R N M N S R
365 gtcactcagctctacatgcagctgctgcacgaggtcctgcgcaag
  V T Q L Y M Q L L H E V L R K
410 cgcgactcctctctggagctggcggcgctgcagaa 444
  R D S S L E L A A L Q

```

Figure 2

Note: only top 8 sequence alignments shown.

BLASTP 2.2.1 [Apr-13-2001]

Reference:

Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schäffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402.

RID: 1002019441-1243-32090

Query=

(146 letters)

Database: nr

764,431 sequences; 242,984,850 total letters

If you have any problems or questions with the results of this search please refer to the [BLAST FAQs](#)

Taxonomy reports

Sequences producing significant alignments:		Score (bits)	E Value
<u>ref NP_004664.1 </u>	angiotensin-like 1 precursor; angiotensin...	144	2e-34
<u>ref NP_036053.1 </u>	angiotensin related protein 2 [Mus muscul...	130	4e-30
<u>dbj BAB27951.1 </u>	(AK011976) putative [Mus musculus]	130	4e-30
<u>gb AAF80364.1 AF159049.1</u>	(AF159049) angiotensin II type 1A ...	129	9e-30
<u>ref NP_036230.1 </u>	angiotensin-like 2 precursor; angiotensin...	127	2e-29
<u>ref NP_082609.1 </u>	RIKEN cDNA 2810039D03 gene [Mus musculus] ...	110	4e-24
<u>ref XP_049490.1 </u>	angiotensin-related protein 5 [Homo sapiens]	68	2e-11
<u>ref NP_114123.1 </u>	angiotensin-related protein 5 [Homo sapie...]	67	5e-11



Figure 3

## Pairwise Scores:

## CLUSTAL W (1.81) Multiple Sequence Alignments

Sequence format is Pearson

Sequence 1:	gi hANGY1	human angiotensinogen Y1	491 aa
Sequence 2:	gi hANGPTL1	human angiotensinogen-like 1	491 aa
Sequence 3:	gi zEST-ANGY1	zebrafish angiotensinogen Y1	148 aa
Sequence 4:	gi hANGPTL2	human angiotensinogen-like 2	493 aa
Sequence 5:	gi mARP2	mouse angiotensinogen-related 2	493 aa
Sequence 6:	gi zfEST-ARP2	zebrafish angiotensinogen-related 2	83 aa
Sequence 7:	gi hANGPTL3	human angiotensinogen-like 3	460 aa
Sequence 8:	gi mANGPTL3	mouse angiotensinogen-like 3	454 aa
Sequence 9:	gi zfANGPTL3	zebrafish angiotensinogen-like 3	458 aa
Sequence 10:	gi hANGPTL4	human angiotensinogen-like 4	406 aa
Sequence 11:	gi mANGPTL4	mouse angiotensinogen-like 4	410 aa
Sequence 12:	gi hARP5	human angiotensinogen-like 5	470 aa
Sequence 13:	gi hANGPTLF	human CTD6 angio-like factor	346 aa

Start of Pairwise alignments

Aligning...

## Your Multiple Sequence Alignment:

593851.334375-349049.aln

## CLUSTAL W (1.81) multiple sequence alignment

```

gi|hANGY1      -----MKTFTWTLGVLFFLLVDTHGCRGGQ--FKIKKINQRRYPRATDGKEEAKKCA 50
gi|hANGPTL1    -----MKTFTWTLGVLFFLLVDTHGCRGGQ--FKIKKINQRRYPRATDGKEEAKKCA 50
gi|zEST-ANGPTL1 ---AMCPQVWMFCWFLCVLWCVRISSSSSR-----QRRQMEAPD-PQRQQQCS 44
gi|hANGPTL2    -----MRPLCVTCWWLGLLAAMGAVAGQEDGFEGTEEGSPREFIYLNRYKRAGESQDKCT 55
gi|mARP2       -----MRPLCMTYWWLGLLATVGAATGPEADVEGTEDGSQREYIYLNRYKRAGESPDKCT 55
gi|zEST-ARP2   -----
gi|hARP5       -----MGKPWLRLALQLLLLLGASWAR-----AGAPRCT 28
gi|hANGPTLF    ----MLKKPLSAVTWLCIFIVAFVSHP-----AWLQKLS 30
gi|hANGPTL3    MFTIKLLLFIIVPLVISSRIDQDNSSFDSD-----LSPEPKSRFAMLDDVKILANGLLQLG 54
gi|mANGPTL3    MHTIKLFLFVVPVLIASRVDPDLSSFDSD-----APSEPKSRFAMLDDVKILANGLLQLG 54
gi|zfANGPTL3   --MLILLWLSSLSTTSAPNSKKSPTAPILITAPPTEARSFAMLDDVRLANGLLQLG 58
gi|hANGPTL4    -----MSGAPTAGAALMLCAATAVLLSAQGGPVQSKSPRFASWDEMNVLAHGLLQLG 52
gi|mANGPTL4    -----MRCAPTAGAALVLCAATAGLLSAQGRPAQPEPPRFASWDEMNVLAHGLLQLG 52

```

```

                                →          coiled-coil domain
gi|hANGY1      YTFVLPEQRITGPICVNTKGQDASTIKDMITRMDLENLKDVLRSQKREIDVLQLVVDVDG 110
gi|hANGPTL1    YTFVLPEQRITGPICVNTKGQDASTIKDMITRMDLENLKDVLRSQKREIDVLQLVVDVDG 110
gi|zEST-ANGPTL1 YTFLIPEQKITGPICAS-RGPSLPDP-ERVTRTDMSELRQTLRSQRREMETLRMMVDVDG 102
gi|hANGPTL2    YTFIVPQQRVTGAICVNSKEPEVLE-NRVHKQELELLNELLKQKQIETLQQLVEVDG 114
gi|mARP2       YTFIVPQQRVTGAICVNSKEPEVHLE-NRVHKQELELLNELLKQKQIETLQQLVEVDG 114
gi|zEST-ARP2   -----
gi|hARP5       YTFVLPPQKFTGAVCWSGPASTRATP-EAANASELAALRMVGRHEELLRELQRLAAADG 87
gi|hANGPTLF    KHKTPAQPOLKAANCCEEVKELKAQVANLSSLLSELNKKQERDWSVVMQVMELESNSK- 89
gi|hANGPTL3    HGLKDFVHKTKGQINDIFQKLNIQDSFYDLSLTSEIKKEEKELRRTTYKLQVKNEEVK 114
gi|mANGPTL3    HGLKDFVHKTKGQINDIFQKLNIQDSFYDLSLTSEIKKEEKELRRTTYKLQVKNEEVK 114
gi|zfANGPTL3   QSLREFVHKTKSQINGIFQKLNVFDRSFYQLSVVTSEIKKEEKELKETTIFLKANNEEIR 118
gi|hANGPTL4    QGLREHAERTRSQLSALERRLSACGSACQGTGEGSTDLPLAPESRVD----- 98
gi|mANGPTL4    HGLREHVERTRGQLGALERRMAACGNACQGPKGKDAPFKDSEDRVPEG----- 100

```

Figure 3 (continued)

	<u>coiled-coil domain</u>	
gi hANGY1	NIVNEVKLLRKESRNMNSRVTLQYMQLLHEIIRKR-----DNSLELSQLENKILNVT	162
gi hANGPTL1	NIVNEVKLLRKESRNMNSRVTLQYMQLLHEIIRKR-----DNSLELSQLENKILNVT	162
gi zEST-ANGPTL1	DMVQEMKLLRKESRNMNSRVTLQYMQLLHEIIRKR-----DSSLELAALQX-----	148
gi hANGPTL2	GIVSEVKLLRKESRNMNSRVTLQYMQLLHEIIRKR-----DNALELSQLENRILNQT	166
gi mARP2	GIVSEVKLLRKESRNMNSRVTLQYMQLLHEIIRKR-----DNALELSQLENRILNQT	166
gi zEST-ARP2	-----	
gi hARP5	AVAGKVRALRKESRGLSARLQQLRAQLQHEAGPGAGPGADLGAEPAAALALLGERVLNAS	147
gi hANGPTLF	RMESRLTDAESKYSEMNNQIDIMQLQAAQTVTQTS-----ADAIYDCSSLYQKNYRIS	142
gi hANGPTL3	NMSLELNSKLESLLLEEKILLQKVKYLEEQLTNLIQ--NQPETPEHPEVTSKTFVEKQD	172
gi mANGPTL3	NMSVELNSKLESLLLEEKALQHKVRALEEQLTNLIL--SPAGAEHPEVTSKSFVEQQD	172
gi zfANGPTL3	NLSLEINSKINNILQERSQLHTKVGGLLEKLGKLSQ--SMMPLEQLQEITALKDVETQE	176
gi hANGPTL4	-----PEVLHSLQTLQKAQN	113
gi mANGPTL4	-----QTPETLQSLQTLQKAQN	117

	<u>coiled-coil domain</u>	
gi hANGY1	TEMLKMATRYRELEVKYASLTDLVNNQSVMITLLEEQLR-IFSRQDTHVSPPLVQVVPQ	221
gi hANGPTL1	TEMLKMATRYRELEVKYASLTDLVNNQSVMITLLEEQLR-IFSRQDTHVSPPLVQVVPQ	221
gi zEST-ANGPTL1	-----	
gi hANGPTL2	ADMLQLASKYKDLEHKYQHLATLAHNQSEIIAQLEEHQCR-VPSARFVQPPPAAPPRVY	225
gi mARP2	ADMLQLASKYKDLEHKFQHLAMLAHNQSEVIAQLEEHQCR-VPAARPMQPPPAAPPRVY	225
gi zEST-ARP2	-----	
gi hARP5	AEAQRAAARFHLQDVKFRELAQLVTQSSLIARLERLCPGGAGGQQQVLPPLVPVVPV	207
gi hANGPTLF	GVYKLPDDDFLGSPE-----	157
gi hANGPTL3	NSIKDLLQTVEDQYKQLNQHSQIKEIENQLRRTSIQEP-----	212
gi mANGPTL3	NSIRELLQSVEEQYKQLSQQHMQIKEIEKQLRKTGIQEPS-----	212
gi zfANGPTL3	RTITDLLRSVKEQHDQNLNYQKIKISLEDKVNVDYTFQDTI-----	216
gi hANGPTL4	SRIQQLFHKVAQQQRHLEKQHLRIQHLQSQFGLLDHKLHD-----	153
gi mANGPTL4	SKIQQLFQKVAQQQRYLSKQNLRIQNLQSQIDLLAPTHLD-----	157

	<u>coiled-coil domain</u>	←	→	*
gi hANGY1	HIPNSQQYTPGLLGGNEIQRDPGYPRDLMPPPDLATSPTKSPFKIPPVTFINEGPFKDCQ			281
gi hANGPTL1	HIPNSQQYTPGLLGGNEIQRDPGYPRDLMPPPDLATSPTKSPFKIPPVTFINEGPFKDCQ			281
gi zEST-ANGPTL1	-----			
gi hANGPTL2	QPPTYNRIIN-----QISTNEIQSDQNLKVLPPPLPTMPTLTSLPSSTDKPSGPPWRDCL			279
gi mARP2	QPPTYNRIIN-----QISTNEIQSDQNLKVLPPSLPTMPALTSLSSTDKPSGPPWRDCL			279
gi zEST-ARP2	-----			
gi hARP5	RLVGSTSDTS-----RMLDPAPEPQRDQTORQOEFMASPMPAGHPAVETKPVGPWQDCA			261
gi hANGPTLF	-----			
gi hANGPTL3	-----EISLSSKPRAPRTTFFLQLEIRNVK-HDGIPAECT			247
gi mANGPTL3	-----ENSLSSKSRAPRTTFFLQLENETENTE-QDDLPADECS			247
gi zfANGPTL3	-----EKPMDLNPEPDPFLYLTNSTNGTKDINDFPADCS			252
gi hANGPTL4	-----HEVAKPARRKRLPEMAQVDPAHNVSRHLRLPRDCQ			189
gi mANGPTL4	-----NGVDKTSRGKKLSKMTQLIGLTSNATHLHRPARDCQ			193

	<u>* fibrinogen-like domain</u>	
gi hANGY1	QAKEAGHSVSGIYMIKPENSNGPMQLWCENSIDPGGWTVIQKRTDGSVNFFRNWENYKKG	341
gi hANGPTL1	QAKEAGHSVSGIYMIKPENSNGPMQLWCENSIDPGGWTVIQKRTDGSVNFFRNWENYKKG	341
gi zEST-ANGPTL1	-----	
gi hANGPTL2	QALDGHDTSSIIYLKVPENTNRLMQVWCDQRHDPGGWTVIQRRLDGSVNFFRNWETKQKQ	339
gi mARP2	QALDGHSTSSIIYLKVPENTNRLMQVWCDQRHDPGGWTVIQRRLDGSVNFFRNWETKQKQ	339
gi zEST-ARP2	-----	
gi hARP5	EARQAGHEQSGVYELRVG--RHVVSVWCEQQLEGGGWTVIQRRQDGSVNFFTTWQHYKAG	319
gi hANGPTLF	-----LEVFCDMETSGGGWTIIQRRKSGLVSYRDWKQYKQKQ	194
gi hANGPTL3	TIYNRGEHTSGMYAIRPSN-SQVFHVYCDVISG-SPWTLIQHRI DGSQNFNETWENYKYG	305
gi mANGPTL3	AVYNRGEHTSGVYTIKPRN-SQGFNVYCDTQSG-SPWTLIQHRKDGSQDNFNETWENYKYG	305
gi zfANGPTL3	EVFTRGQKTSGIYPIKPNQ-SEPFYVYCEITPD-GAATVIQRRDGSVDQDQSWKEYEHG	310
gi hANGPTL4	ELFQVGERQSGLFQIQPLG-SPPFLVNCMTSD-GGWTVIQRRHDSVDFNRPWEAYKAG	247
gi mANGPTL4	ELFQEGERHSGLFQIQPLG-SPPFLVNCMTSD-GGWTVIQRRLNGSVDFNQSWKEYKDG	251

Figure 3 (continued)

		<u>fibrinogen-like domain</u>	
gi hANGY1	FGNIDGEYWLGLENIYMLSNQDNYKLLIELEDWSDKKVYAEYSSFRLEPESEFYRLRLGT	401	
gi hANGPTL1	FGNIDGEYWLGLENIYMLSNQDNYKLLIELEDWSDKKVYAEYSSFRLEPESEFYRLRLGT	401	
gi zEST-ANGPTL1	-----		
gi hANGPTL2	FGNIDGEYWLGLENIYWLTNQGNKYLLVTMEDWGRKVFAYASFRLEPESEYKLRRLGR	399	
gi mARP2	FGNIDGEYWLGLENIYWLTNQGNKYLLVTMEDWGRKVFAYASFRLEPESEYKLRRLGR	399	
gi zEST-ARP2	-----		
gi hARP5	FGRPDGEYWLGLEPVYQLTSRGDHELLVLLLEDWGGRGARAHYDGFSLPESEDHYRLRLGQ	379	
gi hANGPTLF	FGSIRGDEFWLGNENIHRLSRQ-PTLRVEMEDWEGNLRVYAEYSHFVLGNELNSYRLFLGN	253	
gi hANGPTL3	FGRLDGEFWLGLEKIYIVKQSNYVLRLELEDWKNKHYIEYS-FYLGNETNYTLHLVA	364	
gi mANGPTL3	FGRLDGEFWLGLEKIYAIQQSNYILRLELDQWKNKHYIEYS-FHLGSHETNYTLHVAE	364	
gi zfANGPTL3	FGKLEKEFWLGLAKIHSIAQQGEYILHIELEDWKEEKRFIEYT-FTLEGPASDYALHLAP	369	
gi hANGPTL4	FGDPHGEFWLGLEKVHSITGDRNSRLAVQLRDWDGNAELLQFS-VHLGGEDTAYSLQLTA	306	
gi mANGPTL4	FGDPQGEFWLGLEKMSITGDRGSQLAVQLQDWDGNAKLLQFP-IHLGGEDTAYSLQLTE	310	
		<u>fibrinogen-like * domain</u> *	
gi hANGY1	YQG--NAG-DSMMWHNGKQFTTLDRDKDYMAG-NCAHFHKGWWY-NACAHSNLNGVWYR	456	
gi hANGPTL1	YQG--NAG-DSMMWHNGKQFTTLDRDKDYMAG-NCAHFHKGWWY-NACAHSNLNGVWYR	456	
gi zEST-ANGPTL1	-----		
gi hANGPTL2	YHG--NAG-DSFTWHNGKQFTTLDRDHDVYTG-NCAHYQKGGWWY-NACAHSNLNGVWYR	454	
gi mARP2	YHG--NAG-DSFTWHNGKQFTTLDRDHDVYTG-NCAHYQKGGWWY-NACAHSNLNGVWYR	454	
gi zEST-ARP2	-----YSAKQYTTLDRLDHDVYTR-NCAHYQKGGCEL-NACAHSNLNGVPYR	44	
gi hARP5	YHG--DAG-DSLSWHNDKPFSTVDRDRDSYSG-NCALYQKGGWWY-NACAHSNLNGVWHH	434	
gi hANGPTLF	YTG--NVGNDAQYHNNTAFSTKDKDNDNCLD-KCAQLRKGGYWY-NCCTDSNLNGVYYR	309	
gi hANGPTL3	ITG---NVPNAIPENKDLVFST--WDHKAKGHFNCPEGYSGGWWWHDECENNLNGKYNK	419	
gi mANGPTL3	IAG---NIPGALPEHTDLMEST--WNHRAKQLYCPESYSGGWWWHNDICENNLNGKYNK	419	
gi zfANGPTL3	LSG---DLSDAMSNHTGMKFSTKDRDNDNHDESNCARNYTGWWWF-DACGDTNLNGRYAW	425	
gi hANGPTL4	PVAGQLGATTVPSPGLSVPFSTWDQDHDRLRRDKNCAKSLSGGWWWF-GTCSHNLNGQYFR	365	
gi mANGPTL4	PTANELGATNVSPNGLSLPFSTWDQDHDRLRDLNCAKSLSGGWWWF-GTCSHNLNGQYFH	369	
		←	
gi hANGY1	GG-HYRSKHQDGI FWAERYGGSYSRLRAVQMMIKPID-----	491	
gi hANGPTL1	GG-HYRSKHQDGI FWAERYGGSYSRLRAVQMMIKPID-----	491	
gi zEST-ANGPTL1	-----		
gi hANGPTL2	GG-HYRSRYQDGVYWAEFRRGGSYSLLKKVMMIRPNPNTFH-	493	
gi mARP2	GG-HYRSRYQDGVYWAEFRRGGSYSLLKKVMMIRPNPDIFH-	493	
gi zEST-ARP2	GP-HHRRHDHGDGVYCEFRRSAYSLENVEMMILPDPITFH-	83	
gi hARP5	GG-HYRSRYQDGVYWAEFRRGAYSRLKAAAMLIRPLKL----	470	
gi hANGPTLF	LG-EHN-KHLDGITWYGWHSYSLKRVEMKIRPEDFKP--	346	
gi hANGPTL3	PRAKSKPERRRGLSWKSQNGRLYSIKSTKMLIHPTDSESFE	460	
gi mANGPTL3	PRTKSRPERRRGIYWRPQSRKLYAIKSSKMLQPTT-----	454	
gi zfANGPTL3	MRSKARHQRRKGSS-----YTLKSTKITIRPSTHFNNP	458	
gi hANGPTL4	SIPQQRQKLKKGIFWKTWRGRYYPLQATTMLIQPMAAEAAS	406	
gi mANGPTL4	SIPRQRQERKKGIFWKTWKGRYYPLQATTLLIQPMEATAAS	410	

Figure 4

Information for SEQ ID NO: 1

## SEQUENCE CHARACTERISTICS:

- a) LENGTH: 444
- b) TYPE: nucleic acid
- c) STRANDEDNESS:
- d) TOPOLOGY: linear
- e) ORGANISM: *Danio rerio*

MOLECULE TYPE: cDNA

SEQUENCE DESCRIPTION: zebrafish EST similar to Angiopoietin Y1

LOCUS AW174181 444 bp mRNA EST 16-NOV-1999

DEFINITION fi40b08.y1 Sugano Kawakami zebrafish DRA *Danio rerio* cDNA clone 2640087 5' similar to TR:095841 095841 ANGIOPOIETIN Y1. ;, mRNA sequence.

ACCESSION AW174181

VERSION AW174181.1 GI:6440129

KEYWORDS EST.

SOURCE zebrafish.

ORGANISM *Danio rerio*  
 Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Actinopterygii; Neopterygii; Teleostei; Euteleostei; Ostariophysi; Cypriniformes; Cyprinidae; *Danio*.

REFERENCE 1 (bases 1 to 444)

AUTHORS Sugano, S., Kawakami, K., Johnson, S., Li, F., Marra, M., Eddy, S., Hillier, L., Clifton, S., Allen, M., Gibbons, M., Jost, S., Kucaba, T., Martin, J., Pape, D., Steptoe, M., Underwood, K., Theising, B., Ritter, E., Bowers, Y., Wylie, T., Waterston, R. and Wilson, R.

TITLE WashU Zebrafish EST Project 1999

JOURNAL Unpublished (1999)

COMMENT Other ESTs: fi40b08.x1  
 Contact: S.L. Johnson  
 Washington University School of Medicine  
 4444 Forest Park Parkway, Box 8501, St. Louis, MO 63108, USA  
 Tel: 314 286 1800  
 Fax: 314 286 1810  
 Email: [est@watson.wustl.edu](mailto:est@watson.wustl.edu)  
 Library constructed by Dr. Sumio Sugano and Dr. Koichi Kawakami DNA Sequencing by: Washington University Genome Sequencing Center  
 Seq primer: T3 ET from Amersham.

FEATURES Location/Qualifiers

source 1..444  
 /organism="Danio rerio"  
 /strain="AB"  
 /db\_xref="taxon:7955"  
 /clone="2640087"  
 /clone\_lib="Sugano Kawakami zebrafish DRA"  
 /sex="mixed (one male and one female, including unfertilized eggs)"  
 /dev\_stage="adult"  
 /lab\_host="DH10B (phage resistant)"  
 /note="Vector: pME18S-FL3; Site\_1: DraIII (CACTGTGTG);



**Figure 4 (continued)**

Site\_2: DraIII (CACCATGTG); 1st strand cDNA was primed with an oligo(dT) primer [ATGTGGCCTTTTTTTTTTTTTTTTTT]; double-stranded cDNA was ligated to a DraIII adaptor [TGTTGGCCTACTGG], digested and cloned into distinct DraIII sites of the pME18S-FL3 vector (5' site CACTGTGTG, 3' site CACCATGTG). XhoI should be used to isolate the cDNA insert. Size selection was performed to exclude fragments <1.5kb. Library constructed by Dr. Sumio Sugano (University of Tokyo Institute of Medical Science) and kindly donated by Dr. Koichi Kawakami. Custom primers for sequencing: 5' end primer CTTCTGCTCTAAAAGCTGCG and 3' end primer CGACCTGCAGCTCGAGCACA. "

```

BASE COUNT      79 a      126 c      157 g      82 t
ORIGIN
   1  tgctatgtgt cctcaggtct ggatgttctg ctggttcctc tgtgtgctgt ggtgtgtgcg
  61  gatcagcagt tcctccagcc ggcagcgag gcagatggag gctccagacc cgcagcggca
 121  gcagcagtgc tcctacacct tcttaatccc ggagcagaag atcacggggc ctatctgtgc
 181  cagccggggg ccctcgctgc cggaccccga gcgcgtgacg cggacggaca tgtctgagct
 241  gcggcagacc ctgagccggc agcggcgagg gatggagacg ctgcggatga tgggtggatgt
 301  ggacggagac atggtgcagg agatgaagct gctgcgtaag gagtcgcgga acatgaactc
 361  gcgggtcact cagctctaca tgcagctgct gcacgaggtc ctgcgcaagc gcgactcctc
 421  tctggagctg gcggcgctgc agaa

```

Figure 5

Information for SEQ ID NO: 2

## SEQUENCE CHARACTERISTICS:

- a) LENGTH: 2066 bp
- b) TYPE: nucleic acid
- c) STRANDEDNESS:
- d) TOPOLOGY: linear
- e) ORGANISM: Homo sapiens

MOLECULE TYPE: cDNA

SEQUENCE DESCRIPTION: human angiopoietin-like 1 precursor

LOCUS XM\_001720 2066 bp mRNA PRI 27-AUG-2001  
 DEFINITION Homo sapiens angiopoietin-like 1 (ANGPTL1), mRNA.  
 ACCESSION XM\_001720  
 VERSION XM\_001720.2 GI:14724472  
 KEYWORDS  
 SOURCE human.  
 ORGANISM Homo sapiens  
 Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;  
 Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.  
 REFERENCE 1 (bases 1 to 2066)  
 AUTHORS NCBI Annotation Project.  
 TITLE Direct Submission  
 JOURNAL Submitted (23-AUG-2001) National Center for Biotechnology  
 Information, NIH, Bethesda, MD 20894, USA  
 On Jul 16, 2001 this sequence version replaced gi:11423700.

FEATURES  
 Location/Qualifiers  
 source 1..2066  
 /organism="Homo sapiens"  
 /db\_xref="taxon:9606"  
 /chromosome="1"  
 gene 1..2066  
 /gene="ANGPTL1"  
 /note="ANG3; ANGY; ARP1; ANGPT3"  
 /db\_xref="LocusID:9068"  
 /db\_xref="MIM:603874"  
 CDS 434..1909  
 /gene="ANGPTL1"  
 /codon\_start=1  
 /product="angiopoietin-like 1 precursor"  
 /protein\_id="XP\_001720.1"  
 /db\_xref="GI:11423701"  
 /translation="MKTFTWTLGVLFLLVDTGHCRRGGQFKIKKINQRRYPRATDGKE  
 EAKKCAYTFLVPEQRITGPICVNTKGQDASTIKDMITRMDLENLKDVLRSQKREIDVL  
 QLVVDVDGNIVNEVKLLRKESRNMNSRVTQLYMQLLHEIIRKRDNSLELSQLENKILN  
 VTTEMLKMATRYRELEVKYASLTDLVNNQSVMITLLEEQLRIFSRQDTHVSPPLVQV  
 VPQHIPNSQQYTPGLLGGNEIQRDPGYPRDLMPPDLATSPTKSPFKIPPVTFINEGP  
 FKDCQQAKEAGHSVSGIYMIKPENSGPMQLWCENSLDPGGWTVIQKRTDGSVNFFRN  
 WENYKKGFGNIDGEYWLGLENIYMLSNQDNYKLLIELEDWSDKKVYAEYSSFRLEPES  
 EFYRLRLGTYQGNAGDSMMWHNGKQFTTLDRDKDMYAGNCAHFHKGWWYNACAHSNL"

Figure 5 (continued)

NGVWYRGGHYRSKHQDGI FWA EYRGGSYSLRAVQMMIKPID"

misc feature 1262..1903  
/note="FBG; Region: Fibrinogen-related domains (FReDs)"

misc feature 1265..1900  
/note="fibrinogen\_C; Region: Fibrinogen beta and gamma chains"

variation complement(2014)  
/allele="A"  
/allele="C"  
/db\_xref="dbSNP:1544124"

BASE COUNT 720 a 401 c 429 g 516 t

ORIGIN

```

1 atttctccat gtggcagaca gagcaaagcc acaacgcttt ctctgctgga ttaaagacgg
61 cccacagacc agaacttcca ctatactact taaaattaca taggtggctt gtcaaattca
121 attgattagt attgtaaaag gaaaaagaag ttccttctta cagcttggat tcaacggctc
181 aaaacaaaaa tgcagctgcc attaaagtca cagatgaaca aacttctaca ctgattttta
241 aaatcaagaa taagggcagc aagtttctgg attcactgaa tcaacagaca caaaaagctg
301 gcaatatagc aactatgaag agaaaagcta ctaataaaat taacccaacg catagaagac
361 ttttttttct cttctaaaaa caactaagta aagacttaaa tttaaacaca tcatttttaca
421 acctcatttc aaaatgaaga cttttacctg gaccctaggt gtgctattct tcctactagt
481 ggacactgga cattgcagag gtggacaatt caaaattaaa aaaataaacc agagaagata
541 ccctcgtgcc acagatggta aagaggaagc aaagaaatgt gcatacacat tcctggtacc
601 tgaacaaaga ataacagggc caatctgtgt caacaccaag gggcaagatg caagtaccat
661 taaagacatg atcaccagga tggacctga aaacctgaag gatgtgctct ccaggcagaa
721 gcgggagata gatgttctgc aactgggtgg ggatgtagat ggaaacattg tgaatgaggt
781 aaagctgctg agaaaggaaa gccgtaacat gaactctcgt gttactcaac tctatatgca
841 attattacat gagattatcc gtaagaggga taattcactt gaactttccc aactggaaaa
901 caaaatcctc aatgtcacca cagaaatgtt gaagatggca acaagataca gggaactaga
961 ggtgaaatac gcttccttga ctgatcttgt caataaccaa tctgtgatga tcacttttgt
1021 ggaagaacag tgcttgagga tattttcccg acaagacacc catgtgtctc cccacttgt
1081 ccaggtggtg ccacaacata ttcctaacag ccaacagtat actcctggtc tgctgggagg
1141 taacgagatt cagagggatc caggttatcc cagagattta atgccaccac ctgatctggc
1201 aacttctccc accaaaagcc ctttcaagat accaccggta actttcatca atgaaggacc
1261 attcaaagac tgtcagcaag caaaagaagc tgggcattcg gtcagtggga tttatatgat
1321 taaacctgaa aacagcaatg gaccaatgca gttatgggtg gaaaacagtt tggaccttgg
1381 gggttggact gttattcaga aaagaacaga cggctctgtc aacttcttca gaaattggga
1441 aaattataag aaagggtttg gaaacattga cggagaatac tggcttggac tggaaaatat
1501 ctatatgctt agcaatcaag ataattacaa gttattgatt gaattagaag actggagtga
1561 taaaaaagtc tatgcagaat acagcagctt tcgtctggaa cctgaaagtg aattctatag
1621 actgcgctg ggaacttacc agggaaatgc aggggattct atgatgtggc ataatggtaa
1681 acaattcacc aactggaca gagataaaga tatgtatgca ggaaactgcg cccactttca
1741 taaaggaggc tgggtgtaca atgcctgtgc acattctaac ctaaattggag tatggtacag
1801 aggaggccat tacagaagca agcaccaaga tggaaatttc tgggccgaat acagaggcgg
1861 gtcatactcc ttaagagcag ttcagatgat gatcaagcct attgactgaa gagagacact
1921 cgccaattta aatgacacag aactttgtac ttttcagctc ttaaaaatgt aaatgttaca
1981 tgtatattac ttggcacaaat ttatttctac acagaaagtt tttaaaatga attttaccgt
2041 aactataaaa gggaacctat aaatgt

```

Figure 6

Information for SEQ ID NO: 3

## SEQUENCE CHARACTERISTICS:

- a) LENGTH: 1518 bp
- b) TYPE: nucleic acid
- c) STRANDEDNESS:
- d) TOPOLOGY: linear
- e) ORGANISM: Homo sapiens

MOLECULE TYPE: cDNA

SEQUENCE DESCRIPTION: human angiopoietin-like 2

LOCUS	NM_012098	1518 bp	mRNA	PRI	02-NOV-2000
DEFINITION	Homo sapiens angiopoietin-like 2 (ANGPTL2), mRNA.				
ACCESSION	NM_012098				
VERSION	NM_012098.1 GI:6912235				
KEYWORDS	.				
SOURCE	human.				
ORGANISM	Homo sapiens				
	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.				
REFERENCE	1 (bases 1 to 1518)				
AUTHORS	Kim,I., Moon,S.O., Koh,K.N., Kim,H., Uhm,C.S., Kwak,H.J., Kim,N.G. and Koh,G.Y.				
TITLE	Molecular cloning, expression, and characterization of angiopoietin-related protein. angiopoietin-related protein induces endothelial cell sprouting				
JOURNAL	J. Biol. Chem. 274 (37), 26523-26528 (1999)				
MEDLINE	99403103				
PUBMED	10473614				
COMMENT	<p>REVIEWED REFSEQ: This record has been curated by NCBI staff. The reference sequence was derived from <u>AF125175.1</u>.</p> <p>Summary: Angiopoietins are members of the vascular endothelial growth factor family and the only known growth factors largely specific for vascular endothelium. Angiopoietin-1, angiopoietin-2, and angiopoietin-4 participate in the formation of blood vessels. ANGPTL2 protein is a secreted glycoprotein with homology to the angiopoietins and may exert a function on endothelial cells through autocrine or paracrine action.</p>				
FEATURES	Location/Qualifiers				
source	1..1518				
	/organism="Homo sapiens"				
	/db_xref="taxon:9606"				
gene	1..1518				
	/gene="ANGPTL2"				
	/note="ARP2; HARP"				
	/db_xref="LocusID:23452"				
	/db_xref="MIM:605001"				
CDS	22..1503				
	/gene="ANGPTL2"				
	/note="angiopoietin-related protein 2"				
	/codon_start=1				
	/db_xref="LocusID:23452"				



Figure 6 (continued)

```

/db_xref="MIM:605001"
/product="angiopoietin-like 2 precursor"
/protein_id="NP_036230.1"
/db_xref="GI:6912236"
/translation="MRPLCVTCWWLGLLAAMGAVAGQEDGFEGTEEGSPREFIYLNRY
KRAGESQDKCTYTFIVPQQRVTGAICVNSKEPEVLLNENRVHKQELELLNNELLKQKRQ
IETLQQLVEVDGGIVSEVKLLRKESRNMNSRVTQLYMQLLHEIIRKRDNALELSQLEN
RILNQTADMLQLASKYKDLEHKYQHLATLAHNQSEIIAQLEEHQORVPSARVPVQPPP
AAPPRVYQPPTYNRIINQISTNEIQSDONLKVLPPLPTMTLTSLPSSTDKPSGPWR
DCLQALEDGHDTSIIYLKVPENTNRLMQVWCDQRHDPGGWTVIQRRLDGSVNFFRNWE
TYKQGFNIDGEYWLGLENIYWLTNQGNKLLVTMEDWSGRKVFAEYASFRLEPESEY
YKLRLGRYHGNAGDSFTWHNGKQFTTLD RDHDVYTGNC AHYQKGGWYNACAHSNLNG
VWYRGGHYRSRYQDGVYWAEFRGGSYSLLKKVMMIRPNPNTFH"
sig_peptide      22..84
mat_peptide      85..1500
/product="angiopoietin-like 2"
misc_feature      847..1482
/note="FBG; Region: Fibrinogen-related domains (FREds)"
misc_feature      847..1482
/note="fibrinogen_C; Region: Fibrinogen beta and gamma
chains"
BASE COUNT      364 a    469 c    433 g    252 t
ORIGIN
    1 aaccaccatt ttgcaaggac catgaggcca ctgtgcgtga catgctggtg gctcggactg
   61 ctggctgcca tgggagctgt tgcaggccag gaggacggtt ttgagggcac tgaggagggc
  121 tcgccaagag agttcattta cctaaacagg tacaagcggg cgggcgagtc ccaggacaag
  181 tgcacctaca ccttcattgt gccccagcag cgggtcacgg gtgccatctg cgtcaactcc
  241 aaggagcctg aggtgcttct ggagaaccga gtgcataagc aggagctaga gctgctcaac
  301 aatgagctgc tcaagcagaa gcggcagatc gagacactgc agcagctggt ggaggtggac
  361 ggcggcattg tgagcgaggt gaagctgctg cgcaaggaga gccgcaacat gaactcgcgg
  421 gtcacgcagc tctacatgca gctcctgcac gagatcatcc gcaagcggga caacgcgttg
  481 gagctctccc agctggagaa caggatcctg aaccagacag ccgacatgct gcagctggcc
  541 agcaagtaca aggacctgga gcacaagtac cagcacctgg ccacactggc ccacaaccaa
  601 tcagagatca tcgcgcagct tgaggagcac tgccagaggg tgccctcggc caggcccgtc
  661 ccccagccac ccccgcgtgc cccgccccgg gtctaccaac caccaccta caaccgcac
  721 atcaaccaga tctctaccaa cgagatccag agtgaccaga acctgaaggt gctgccaccc
  781 cctctgcccc ctatgcccac tctcaccage ctcccatctt ccaccgaaa gccgtcgggc
  841 ccatggagag actgcctgca ggccctggag gatggccacg acaccagctc catctacctg
  901 gtgaagccgg agaacaccaa ccgcctcatg caggtgtggt gcgaccagag acacgacccc
  961 gggggctgga ccgtcatcca gagacgcctg gatggctctg ttaacttctt caggaactgg
 1021 gagacgtaca agcaagggtt tgggaacatt gatggcgaat actggctggg cctggagAAC
 1081 atttactggc tgacgaacca aggcaactac aaactcctgg tgaccatgga ggactggtcc
 1141 ggccgcaaag tctttgcaga atacgccagt ttccgcctgg aacctgagag cgagtattat
 1201 aagctgcggc tggggcgcta ccatggcaat gcgggtgact cctttacatg gcacaacggc
 1261 aagcagttca ccaccctgga cagagatcat gatgtctaca caggaaactg tgcccactac
 1321 cagaagggag gctggtggtg taacgcctgt gccactcca acctcaacgg ggtctggtac
 1381 cgcggggggc attaccggag ccgctaccag gacggagtct actgggctga gttccgagga
 1441 ggctcttact cactcaagaa agtggtgatg atgatccgac cgaaccccaa caccttcac
 1501 taagccagct cccctcc

```

## Figure 7

Information for SEQ ID NO: 4

## SEQUENCE CHARACTERISTICS:

- a) LENGTH: 1518 bp
- b) TYPE: nucleic acid
- c) STRANDEDNESS:
- d) TOPOLOGY: linear
- e) ORGANISM: *Mus musculus*

MOLECULE TYPE: cDNA

SEQUENCE DESCRIPTION: mouse angiopoietin-related protein-2

LOCUS	AF125176	1518 bp	mRNA	ROD	16-SEP-1999
DEFINITION	Mus musculus angiopoietin-related protein-2 mRNA, complete cds.				
ACCESSION	AF125176				
VERSION	AF125176.1 GI:5901652				
KEYWORDS	.				
SOURCE	house mouse.				
ORGANISM	<i>Mus musculus</i> Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Mus.				
REFERENCE	1 (bases 1 to 1518)				
AUTHORS	Kim, I., Moon, S.O., Koh, K.N., Kim, H., Uhm, C.S., Kwak, H.J., Kim, N.G. and Koh, G.Y.				
TITLE	Molecular cloning, expression, and characterization of angiopoietin-related protein. angiopoietin-related protein induces endothelial cell sprouting				
JOURNAL	J. Biol. Chem. 274 (37), 26523-26528 (1999)				
MEDLINE	99403103				
PUBMED	10473614				
REFERENCE	2 (bases 1 to 1518)				
AUTHORS	Kim, I., Moon, S.O. and Koh, G.Y.				
TITLE	Direct Submission				
JOURNAL	Submitted (02-FEB-1999) Cardiac Regeneration Group, National Research Initiatives, Chonbuk University, San 2-20, Keum-Am-Dong, Chonju 560-180, Korea				
FEATURES	Location/Qualifiers				
source	1..1518 /organism="Mus musculus" /db_xref="taxon:10090" /tissue_type="heart" /dev_stage="adult"				
CDS	22..1503 /note="contains N-terminal coiled-coil domain and C-terminal fibrinogen-like domain" /codon_start=1 /product="angiopoietin-related protein-2" /protein_id="A05358.1" /db_xref="GI:5901653" /translation="MRPLCMTYWWLGLLATVGAATGPEADVEGTEDGSQREYIYLNRY KRAGESPDKCTYTFIVPQQRVTGAICVNSKEPEVHLENRVHKQELELLNNELLKQKRQ IETLQQLVEVDGGIVSEVKLLRKESRNMNSRVTQLYMQLLHEIIRKRDNALELSQLEN RIINQTADMLQLASKYKDLEHKFQHLAMLAHNQSEVIAQLEEHQCRVPAARFMPQPPP				

Figure 7 (continued)

AAPPRVYQPPTYNRIINQISTNEIQSDQNLKVLPPSLPTMPALTSLPSSTDKPSGPWR  
 DCLQALEDDGHSTSSIYLVKPEENTNRLMQVWCDQRHDPGGWTVIQRRLDGSVNFFRNWE  
 TYKQGFNIDGEYWLGLENIYWLTNQGNKLLVTMEDWSGRKVFAEYASFRLEPESEY  
 YKLRLGRYHGNAGDSFTWHNGKQFTTLDRDHDVYTGNCAYQKGGWWYNACAHSNLNG  
 VWYRGGHYRSRYQDGVYWAEEFRGGSYSLLKKVMMIRPNPDIFH"

BASE COUNT	383 a	427 c	421 g	287 t	
ORIGIN					
1	aaccagcaca	ctgcaaggac	catgaggcca	ctgtgtatga	cctactgggtg gcttggactg
61	ctggccacgg	tcggagctgc	tacaggccca	gaggctgacg	ttgagggcac agaggatggt
121	tcacagagag	agtacattta	cctcaacagg	tacaagcggg	caggtgagtc ccccgacaag
181	tgcacctaca	ctttcattgt	gccccagcag	cgggtcacag	gtgccatttg tgtcaactcc
241	aaggagcctg	aggtgcacct	ggagaaccgt	gtgcacaage	aggagctgga gctgctcaac
301	aatgagctgc	ttaagcagaa	gcggcagatc	gagacgctgc	agcagctggt agaggtagac
361	ggaggcatcg	tgagcgaggt	gaagctgctg	cgcaaggaga	gccgcaacat gaactcgaga
421	gtcacgcagc	tgtacatgca	acttctacat	gagatcattc	gaaagcgaga caatgcgctg
481	gagctctccc	agctggagaa	caggatcctg	aaccagacag	ctgacatgct gcagctggct
541	agcaagtaca	aggacctgga	gcacaagtgc	cagcacctgg	ctatgctggc acacaacca
601	tcagaggtca	ttgctcagct	cgaagagcac	tgccaacgcg	tacctgcagc caggcctatg
661	ccccagccac	ccccagcage	tccacctcgg	gtctaccaac	caccaccta caaccgcac
721	atcaaccaga	tttccacca	tgagatccag	agtgaccaga	atctgaaggt gctgcccgcc
781	tccttgccca	ccatgcctgc	ccttaccagt	ctcccatctt	ccactgataa gccatcaggt
841	ccatggagag	actgcctgca	agccctggaa	gatggtcaca	gcaccagctc catctacctg
901	gtgaagcctg	agaataccaa	ccgcctgatg	caggtgtggt	gtgaccagag acatgaccct
961	ggagggttga	ctgtcatcca	gagacgcctg	gatggctctg	tcaacttctt caggaactgg
1021	gagacctata	agcaagggtt	tgggaacatc	gatggtgagt	actggctggg cctggagaac
1081	atctactggc	tgacgaacca	aggcaactac	aaactgctgg	taaccatgga ggactggtct
1141	ggccgtaaag	tctttgctga	gtatgccagt	ttccgactgg	agccagaaag cgagtactat
1201	aagctgcggc	tggggcggtta	tcattggcaat	gcaggcgact	cctttacctg gcacaacggc
1261	aaacagttta	ccaccctgga	cagggaccat	gatgtctaca	caggaaactg tgcccactat
1321	cagaaggag	gatggtggtta	taacgcctgt	gctcaetcca	acctcaatgg ggtctggtac
1381	cgtggggggc	attaccggag	cagataccag	gacgggggtct	actgggctga gttccgagga
1441	ggctcttact	cactcaagaa	ggtggtgatg	atgattcggc	ccaaccccga tatcttccac
1501	taagctctcc	ctgcctgg			

**Figure 8**

**a) Information for SEQ ID NO: 5**

**SEQUENCE CHARACTERISTICS:**

- a. LENGTH: 25 bp
- b. TYPE:
- c. STRANDEDNESS: single
- d. TOPOLOGY: linear
- e. ORGANISM: Danio rerio

**MOLECULE TYPE:** antisense oligomer

**SEQUENCE DESCRIPTION:** antisense nucleotide sequence against zebrafish angiopoietin-like 1 precursor

5'ACATCCAGACCTGAGGACACATAGC3'

**b) Information for SEQ ID NO: 6**

**SEQUENCE CHARACTERISTICS:**

- a. LENGTH: 25 bp
- b. TYPE:
- c. STRANDEDNESS: single
- d. TOPOLOGY: linear
- e. ORGANISM: Danio rerio

**MOLECULE TYPE:** antisense oligomer

**SEQUENCE DESCRIPTION:** antisense nucleotide sequence against zebrafish angiopoietin

Y1

5'ATGGCTTTCTGGGATGCGACTCAGG3'



## Figure 9

a)

Information for SEQ ID NO: 7

## SEQUENCE CHARACTERISTICS:

- a. LENGTH: 491
- b. TYPE: amino acid
- c. STRANDEDNESS:
- d. TOPOLOGY:
- e. ORGANISM: Homo sapiens

MOLECULE TYPE: Protein

SEQUENCE DESCRIPTION: human angiopoietin-like 1 precursor

MKTFTWTLGVLFLLVDTGHCRCGGQFKIKKINQRRYPRTDGGKEEAKKCAYTFLVPEQRITGPICVNTKG  
 QDASTIKDMITRMDLENLKDVLRSQKREIDVLQLVVDVDGNIVNEVKLLRKESRNMNSRVTQLYMQLLHE  
 IIRKRDNSLELSQLENKILNVTTEMLKMATRYRELEVKYASLTDLVNNQSVMITLLEEQLRIFSRQDTH  
 VSPPLVQVVPQHIPNSQQYTPGLLGGNEIQRDPGYPRDLMPPPDLATSPKSPFKIPPVTFINEGPFKDC  
 QQAKEAGHSVSGIYMIKPENSNQPMQLWCENSLDPGGWTVIQKRTDGSVNFNWNENYKKGFGNIDGEYW  
 LGLENIYMLSNQDNYKLLIELEDWSDKKVYAEYSSFRLEPESEFYRLRLGTYQGNAGDSMMWHNGKQFTT  
 LDRDKDMYAGNCAHFHKGWWYNACAHSNLNGVWYRGGHYRSKHQDGIFWAEYRGGSYSLRAVQMMIKPI  
 D

b)

Information for SEQ ID NO: 8

## SEQUENCE CHARACTERISTICS:

- a. LENGTH: 493
- b. TYPE: amino acid
- c. STRANDEDNESS:
- d. TOPOLOGY:
- e. ORGANISM: Homo sapiens

MOLECULE TYPE: protein

SEQUENCE DESCRIPTION: human angiopoietin-like 2 precursor

MRPLCVTCWWLGLLAAMGAVAGQEDGFEGTEEGSPREFIYLNRYKRAGESQDKCTYTFIVPQQRVTGAIC  
 VNSKEPEVLLENRVHVKQELELLNNELLKQKRQIETLQQLVEVDGGIVSEVKLLRKESRNMNSRVTQLYMQ  
 LLHEIIRKRDNALELSQLENRIINQADMLQLASKYKDLEHKYQHLATLAHNQSEIIAQLEEHQCRVPSA  
 RPVPQPPPAAPPRVYQPPTYNRIINQISTNEIQSDQNLKVLPPPLPTMPTLTSLPSSTDKPSGPWRDCLQ  
 ALEDGHDTSIIYLVKPENTNRLMQVWCDQRHDPGGWTVIQRRLDGSVNFNWNWETKQGFNIDGEYWL  
 LENIYWLTNQGNKLLVTMEDWSGRKVFAEYASFRLEPESEYKLRRLGRYHGNAGDSFTWHNGKQFTTLD  
 RDHDVYTGNCAYHKGWWYNACAHSNLNGVWYRGGHYRSRYQDGVYWAEFRGGSYSLKKVMMIRPNPN  
 TFH

Figure 10

a)

Information for SEQ ID NO: 9

SEQUENCE CHARACTERISTICS:

- a) LENGTH: 8
  - b) TYPE: amino acid
  - c) STRANDEDNESS:
  - d) TOPOLOGY:
  - e) ORGANISM: homo sapiens
- MOLECULE TYPE: protein
- SEQUENCE DESCRIPTION:  $\text{Ca}^{++}$ -binding motif

D/N-K/H-D-V/M-Y-A/T-G/R-N

b)

Information for SEQ ID NO: 10

SEQUENCE CHARACTERISTICS:

- a) LENGTH: 12
  - b) TYPE: amino acid
  - c) STRANDEDNESS:
  - d) TOPOLOGY:
  - e) ORGANISM: homo sapiens
- MOLECULE TYPE: protein
- SEQUENCE DESCRIPTION: fibrinogen beta and gamma chains C-terminal domain

W/C-W/E-Y/L-N-A-C-A-H-S-N-L-N

c)

Information for SEQ ID NO: 11

SEQUENCE CHARACTERISTICS:

- a) LENGTH: 21
  - b) TYPE: amino acid
  - c) STRANDEDNESS:
  - d) TOPOLOGY:
  - e) ORGANISM: homo sapiens
- MOLECULE TYPE: protein
- SEQUENCE DESCRIPTION: secretory signal sequence

M-K-T-F-T-W-T-L-G-V-L-F-F-L-L-V-D-T-G-H-C

Figure 11













Developmental stage in days post-fertilization (dpf)	type of treatment	working morpholino conc. in $\mu\text{M}$	# normal	# less stained	# more stained	Blood Staining
2dpf	zf-ANGPTY1 MO injected	40 $\mu\text{M}$	26	1	-	
	zf-ANGPTY1 MO injected	100 $\mu\text{M}$	-	24	-	
	zf-ANGPTY1 MO injected	200 $\mu\text{M}$	-	28	-	
	wt	-	22	-	-	
3dpf	zf-ANGPTY1 MO injected	40 $\mu\text{M}$	22	3	1	
	zf-ANGPTY1 MO injected	100 $\mu\text{M}$	1	24	-	
	zf-ANGPTY1 MO injected	200 $\mu\text{M}$	-	21	-	
	wt	-	24	-	-	




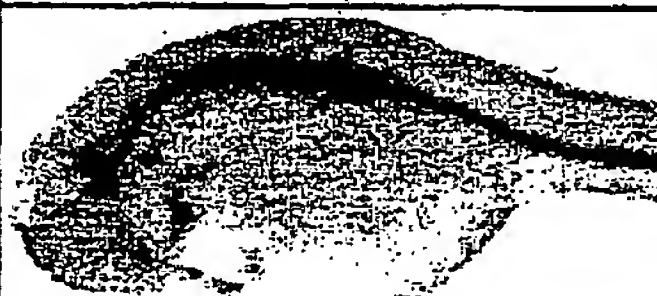
Figure 12

(a)



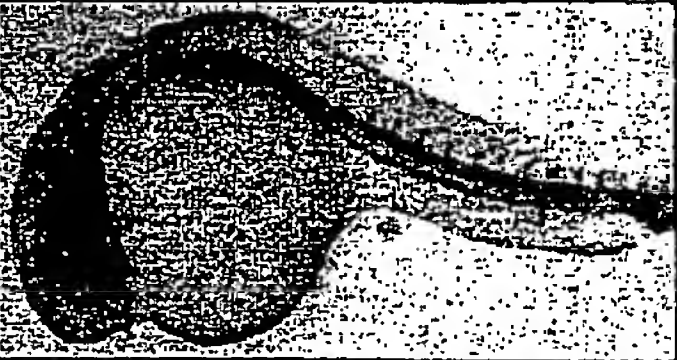

Developmental stage in hours post-fertilization (hpf)	type of treatment	working morpholino conc. in $\mu$ M	# normal	# less stained	# deformed	GATA1 Expression
18 somite	zf-GATA1 MO injected	40 $\mu$ M	23	-	-	not shown
	zf-GATA1 MO injected	100 $\mu$ M	28	-	-	not shown
	Zf-GATA1 MO injected	200 $\mu$ M	29	1	-	not shown
	wt	-	17	-	-	not shown
24hpf	Zf-GATA1 MO injected	40 $\mu$ M	20	3	-	
	Zf-GATA1 MO injected	100 $\mu$ M	23	4	-	
	Zf-GATA1 MO injected	200 $\mu$ M	-	28	-	
	wt	-	14	-	-	







b)

Developmental stage in hours post-fertilization (hpf)	Type of treatment	working morpholino conc. in $\mu$ M	# normal	# less stained	# deformed	Flt-4 Expression
18 somite	zf-Flt-4 MO injected	40 $\mu$ M	25	4	-	not shown
	zf-Flt-4 MO injected	100 $\mu$ M	7	(20)	-	not shown
	zf-Flt-4 MO injected	200 $\mu$ M	15	(11)	-	not shown
	wt	-	21	-	-	not shown
24hpf	zf-Flt-4 MO injected	40 $\mu$ M	18	4	-	
	zf-Flt-4 MO injected	100 $\mu$ M	28	1	-	
	zf-Flt-4 MO injected	200 $\mu$ M	31	2	(1)	
	wt	-	14	-	-	




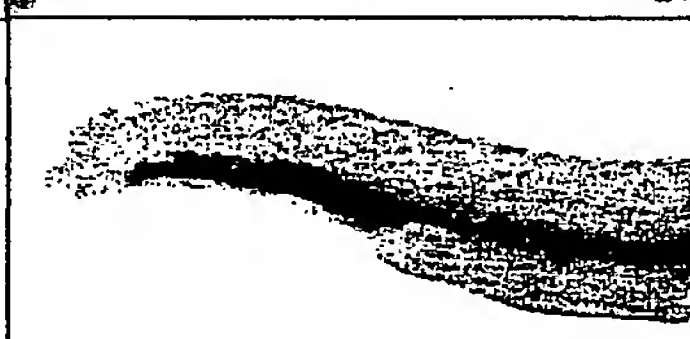
c)

Developmental stage in hours post-fertilization (hpf)	Type of treatment	working morpholino conc. in $\mu\text{M}$	# normal	# less stained	# deformed	Flk-1 Expression
18 somite	zf-Flk-1 MO injected	40 $\mu\text{M}$	26	2	-	not shown
	zf-Flk-1 MO injected	100 $\mu\text{M}$	11	18	-	not shown
	zf-Flk-1 MO injected	200 $\mu\text{M}$	17	9	-	not shown
	wt	-	15	-	-	not shown
24hpf	zf-Flk-1 MO injected	40 $\mu\text{M}$	24	3	-	
	zf-Flk-1 MO injected	100 $\mu\text{M}$	27	-	-	
	zf-Flk-1 MO injected	200 $\mu\text{M}$	27	3	-	
	wt	-	18	-	-	

d)

Developmental stage in hours post-fertilization (hpf)	Type of treatment	working morpholino conc. in $\mu\text{M}$	# normal	# less stained	# deformed	Fli-1 Expression
18 somite	zf-Fli-1 MO injected	40 $\mu\text{M}$	26	-	-	not shown
	zf-Fli-1 MO injected	100 $\mu\text{M}$	10	19	-	not shown
	zf-Fli-1 MO injected	200 $\mu\text{M}$	21	4	-	not shown
	wt	-	21	-	-	not shown
24hpf	zf-Fli-1 MO injected	40 $\mu\text{M}$	24	2	-	
	zf-Fli-1 MO injected	100 $\mu\text{M}$	25	2	-	
	zf-Fli-1 MO injected	200 $\mu\text{M}$	30	-	-	
	wt	-	15	-	-	

e)

Developmental stage in hours post-fertilization (hpf)	Type of treatment	working morpholino conc. in $\mu\text{M}$	# normal	# less stained	# deformed	Lmo-2 Expression
18 somite	zf-lmo-2 MO injected	40 $\mu\text{M}$	30	2	-	not shown
	zf-lmo-2 MO injected	100 $\mu\text{M}$	33	-	-	not shown
	zf-lmo-2 MO injected	200 $\mu\text{M}$	22	7(head)	-	not shown
	wt	-	15	-	-	not shown
24hpf	zf-lmo-2 MO injected	40 $\mu\text{M}$	22	3	-	
	zf-lmo-2 MO injected	100 $\mu\text{M}$	23	4	-	
	zf-lmo-2 MO injected	200 $\mu\text{M}$	26	4	-	
	wt	-	14	-	-	





European Patent  
Office

# EUROPEAN SEARCH REPORT

Application Number  
EP 02 02 1286

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	EP 0 962 530 A (ELI LILLY AND CO ) 8 December 1999 (1999-12-08) SEQ ID NO:2 * page 2, line 49 - page 4, line 16 * * page 12, line 13 - line 18 * *parts "Vectors", "Therapeutic applications" *claims*	1-24	C12N15/11 C12N15/12 C12N5/10 A01K67/027 C07K14/515 C12N15/63 G01N33/50 A61K38/17 A61P7/06 A61P35/02
X	EP 0 995 759 A (ELI LILLY AND CO) 26 April 2000 (2000-04-26) *SEQ ID NO: 2* * page 2, line 46 - page 3, line 56 * * page 13, line 43 - line 47 * * page 17, line 23 - line 32 * *part "Vectors"* *claims*	1-24	
X	WO 99 40193 A (ZYMOGENETICS INC) 12 August 1999 (1999-08-12) *SEQ ID NO:2* * page 43, line 6 - page 46, line 29 * * page 40, line 6 - page 41, line 2 * * page 37, line 23 - page 38, line 2 * * page 36, line 9 - line 18 * * page 33, line 21 - page 34, line 16 * * page 17, line 21 - page 18, line 12 * * page 26, line 8 - page 29, line 23 * * example 2 * *claims*	1-10, 15-42	TECHNICAL FIELDS SEARCHED (Int.Cl.7)  C12N C07K A01N A61K A61P G01N
The present search report has been drawn up for all claims			
Place of search <b>MUNICH</b>		Date of completion of the search <b>5 February 2003</b>	Examiner <b>Böhmerova, E</b>
<p><b>CATEGORY OF CITED DOCUMENTS</b></p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons &amp; : member of the same patent family, corresponding document</p>			

EPO FORM 1503 03 82 (P04C01)



European Patent  
Office

## EUROPEAN SEARCH REPORT

Application Number  
EP 02 02 1286

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	WO 00 59938 A (GENETICS INST) 12 October 2000 (2000-10-12)  *SEQ ID NOs: 9,14* * page 20, line 13 - line 16 * * page 7, line 13 - page 10, line 14 * * page 13, line 27 - page 14, line 24 * * page 18, line 17 - line 32 * *parts "Angiogenic activity of AR2 and AR3", "Cytokine and cell Proliferation/Differentiation Activity", "Hematopoiesis Regulating Activity" * claims 1-46 * -----	1-10, 15-21, 26-42	
			TECHNICAL FIELDS SEARCHED (Int.Cl.7)
The present search report has been drawn up for all claims			
Place of search <b>MUNICH</b>		Date of completion of the search <b>5 February 2003</b>	Examiner <b>Böhmerova, E</b>
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			

EPO FORM 1503 03 82 (P04C01)

**ANNEX TO THE EUROPEAN SEARCH REPORT  
ON EUROPEAN PATENT APPLICATION NO.**

EP 02 02 1286

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report.  
The members are as contained in the European Patent Office EDP file on  
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

05-02-2003

Patent document cited in search report		Publication date		Patent family member(s)	Publication date
EP 0962530	A	08-12-1999	AU	4320499 A	20-12-1999
			EP	0962530 A2	08-12-1999
			WO	9962925 A1	09-12-1999
-----					
EP 0995759	A	26-04-2000	AU	5210899 A	14-02-2000
			EP	0995759 A1	26-04-2000
			WO	0005241 A1	03-02-2000
-----					
WO 9940193	A	12-08-1999	AU	2493699 A	23-08-1999
			WO	9940193 A1	12-08-1999
-----					
WO 0059938	A	12-10-2000	AU	4066800 A	23-10-2000
			EP	1171459 A1	16-01-2002
			WO	0059938 A1	12-10-2000
-----					